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Beetles Infesting Stored Grain in the Willamette Valley of Oregon[1]

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The Willamette Valley has a climatic type distinctly different from that of Oregon's larger grain-producing areas east of the Cascade Mountains. The writers were obligated, as part of a regional project on stored grain insects, to initiate ecological studies of stored grain insects under Willamette Valley climatic conditions. Since little information collected in a systematic and comparative manner is available concerning insect infestations in stored grain in the Willamette Valley, it was necessary first of all to learn which insect species appeared to be most important. Consequently, a survey was made to determine the nature of the stored grain insect problem in this area.

The climate of the Willamette Valley is classified as a marine west coast type, but differs from the typical marine west coast climate in normally having hot, dry summers. In normal years, the moisture content of wheat at harvest time is usually 11% or less. In occasional years, when wet weather occurs at harvest time, the moisture content of wheat may be as high as 17 to 18%. Wheat harvested in the Willamette Valley has a moisture content higher than that from the Oregon wheat-growing areas east of the Cascade Mountains where the average is about 9%[3]. In the Willamette Valley, the moisture content of the surface grain frequently increases considerably during the storage period because of high atmospheric humidity.

Methods.—All samples were taken from bulk grain, including wheat, oats, barley and, occasionally, shelled corn. The samples were 1 gallon amounts, taken from the top foot of grain in most cases. To check on the use of surface samples as a method of sampling, numerous probe samples were taken in 12 infested bins. These probe samples were taken at depths ranging from 3 to 18 feet. The number of insects found decreased sharply with increasing depth. No species were found which did not also occur in the surface samples, nor was there any indication that the species occurred in markedly different proportions at the lower depths. Samples were taken from one or two infested bins in each structure. When referring to a specific infestation or group of infestations, the term "infestation" is used to indicate an infested bin.

The survey reported herein was conducted during the fall, winter and spring months over a 2-year period. Storage units were selected so as to include all parts of the Willamette Valley. About half of the samples were collected during the first year and the other half during the second year. The first year, adult insects were removed from the samples and counted on the day the samples were taken or on the following day. The samples were then discarded. The second year the adults insects were again removed and counted. The living larvae and dust were returned to the samples which were then held for 30 days at 75° F. to allow development of the free-living larvae and of larvae within the kernels. After 30 days, adult insects were removed and counted and the samples discarded. In nearly all of the second-year samples, adult insects were found in the grain after it had been held for 30 days. However, only rarely was a species found after 30 days which was not found initially, nor was the degree of abundance as indicated in table 1 changed appreciably. Therefore, the data for both years are combined in table 1.

Table 1.—Frequency and abundance of beetles found in 89 insect infestations in stored grain in the Willamette Valley of Oregon.

Species	Number of Infestations in Following Categories (per Gallon Sample)			
	Less than 100	100–1000	More than 1000	Total Times Found
Ahasverus advena (Waltl.) Foreign grain beetle	14	3		17
Anthicus floralis (L.)	4			4
Cartodere constricta (Gyll.)	1			1
Corticaria sp.	2			2
Cryptophagus sp.	1			1
Laemophloeus sp. Flat grain beetles	27	7	1	35
Oryzaephilus surinamensis (L.) (Saw-toothed grain beetle)	48	19	3	70
Palorus ratzeburgi Wissman	1			1
Rhyzopertha dominica (F.) Lesser grain borer	1			1
Sitophilus granarius (L.) Granary weevil	26	2	1	29
Sitophilus oryza (L.) Rice weevil	19	5	2	26
Tenebroides mauritanicus (L.) Cadelle	2			2
Tribolium castaneum (Hbst.) Red flour beetle	26	7	2	35
Tribolium confusum (Duv.) Confused flour beetle	1			1
Typhaea stercorea (L.)	12	1		31

Of the 89 infestations referred to in table 1, 45 were in country elevators which stored grain pending removal to terminal elevators and for local use as livestock feed.

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[3] Statements concerning moisture content of wheat are based upon personal communications from E. P. Walker of Crown Mills, Portland, Oregon and D. D. Hill of Oregon State College.

Thirty-three of the infestations were found in farm-stored grain. Of these, 22 were in grain being stored pending marketing and 11 in grain kept for use as feed. The remaining 11 infestations were from miscellaneous types of storage. No record was kept of the number of storage structures where insects were not found since the object of the survey was to determine the insect species present in stored grain in the area and their relative abundance when grain conditions and other environmental factors permitted the development of infestations.

RESULTS.—Table 1 shows the species of beetles collected and their relative abundance as indicated by arbitrarily selected categories. The saw-toothed grain beetle was by far the most common and most abundant species. *Tribolium castaneum* and *Laemophloeus* spp. were also very common. The *Laemophloeus* were not identified to species because of the present unsatisfactory taxonomic status of the North American representatives of the genus. Insect pests of stored grain are commonly divided into primary pests which can feed on sound undamaged kernels and secondary pests which attack only damaged kernels (e.g., Linsley & Michelbacher 1943). *Tribolium castaneum* and *Laemophloeus* spp. are ordinarily considered secondary pests. However, Birch (1947) found that *T. castaneum* and *T. confusum* can reproduce in wheat consisting of only sound uncracked kernels. Daniels (1956) found that damage and reproduction by *T. confusum* and *T. castaneum* in whole kernel wheat increased with increasing moisture content over a range from 10.2% to 14.2%. Lucas & Oxley (1946) have presented evidence that *Laemophloeus* spp. can be primary pests under field conditions. Laboratory studies (Lucas & Oxley 1946) have shown that *L. minutus* "may pass part or nearly the whole of its life history within wheat grains."

Of the 89 infested bins, the granary weevil was present in 29 and the rice weevil in 26. There were 45 infestations in which one or both species were present. As many as 2800 weevils were found in a gallon sample. Prior to the inception of this survey, the rice weevil was assumed to be neither abundant nor widely spread in the Willamette Valley. The combined numbers of the flat grain beetle, saw-toothed grain beetle and red flour beetle sometimes exceeded 6000 per gallon sample. Although these insects may not have initiated the damage, such large populations certainly increased the resulting loss.

The lesser grain borer was first reported in Oregon in 1956 (Goeden & Capizzi 1956). Prior to their report, specimens had been collected from cargoes in Portland, but no infestations were known to be established in Oregon.

Very few moths were found in bulk grain. In the 89 infested bins, the Mediterranean flour moth (*Angasta kühniella*) was found in one, the Indian meal moth (*Plodia interpunctella* (Hbn.)) in three, and *Endrosis lactella* in one.

Although the climate of the Willamette Valley is frequently compared with that of England, insect infestations in grain do not die out during the winter to the extent indicated by Oxley & Howe (1944) in England.

REFERENCES CITED

Birch, L. C. 1947. The ability of flour beetles to breed in wheat. Ecology **28**: 322–4.

Daniels, N. E. 1956. Damage and reproduction by the flour beetles, *Tribolium confusum* and *T. castaneum*, in wheat at three moisture contents. Jour. Econ. Ent. **49**: 244–7.

Goeden, K. J., and J. Capizzi, Jr. 1956. Cooperative Econ. Insect. Rept. U.S. Dept. Agric. **6**: 250.

Linsley, E. G., and A. E. Michelbacher. 1943. A report on insect infestation of stored grain in California. Jour. Econ. Ent. **36**: 829–31.

Lucas, T. E., and T. A. Oxley. 1946. Study of an infestation by *Laemophloeus* sp. (Coleoptera, Cucujidae) in bulk wheat. Ann. Appl. Biol. **33**: 289–93.

Oxley, T. A., and R. W. Howe. 1944. Factors influencing the course of an insect infestation in bulk wheat. Ann. Appl. Biol. **31**: 76–80.

Probable Origin and Dissemination of a Polyhedrosis Virus of an Armyworm in Hawaii[1]

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The rather sudden appearance of a polyhedrosis virus infection in an armyworm, *Spodoptera mauritia* (Bdv.), a recent immigrant to Hawaii, has aroused some interesting speculations on the origin and mode of dissemination of the virus. Did the virus enter Hawaii with the insect host? How was the virus disseminated to widely separated areas from the original site of introduction?

Spodoptera mauritia was first reported on the island of Oahu, Territory of Hawaii, on the basis of adults caught in a light trap in December, 1953 (Pemberton 1955). The light trap, which had been in operation for several years, was located at Barber's Point, approximately 16 miles west of the center of Honolulu. Pemberton surmised that the species was possibly brought to Hawaii in an airplane from Guam or Fiji. The following fall, September, 1954, the first outbreak of this armyworm was observed on lawns planted with Bermuda grass (*Cynodon dactylon* (L.) Persoon) at Wailupe Circle, Honolulu, approximately 23 miles east of Barber's Point (Tanada 1955). During 1954, the armyworm caused severe damage to many lawns in certain parts of Honolulu, especially in the relatively dry Wailupe Circle and adjacent areas, and at Kailua and Kaneohe, recent housing subdivisions on the windward or northeast section of the island.

In February, 1956, slightly more than 2 years after the

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first discovery of *S. mauritia* in Hawaii, several individuals of this armyworm were found infected with a polyhedrosis virus (Bianchi, unpub.). A survey was conducted during the next 2 months throughout the armyworm-affected areas to determine the distribution of the virus among larvae in different armyworm populations on the island of Oahu. Larvae infected with virus were found in 11 different populations in lawns located at four housing subdivisions—Waialae-Kahala, Wailupe Circle, Aina Haina, and Niuiki Circle (fig. 1). About half of these populations consisted of young armyworms of the first to fourth instars, and the other half of older armyworms in the fifth to last instars. The populations of older armyworms had caused some damage to lawns and were discovered after the virus epizootic had started to decline. The greatest distance separating infected armyworm populations was about 3¼ miles. No virus-infected larvae were found on lawns in the windward areas of Oahu.

Since the female moths tend to lay their eggs predominantly on walls of buildings and on leaves of trees and shrubs, the armyworm probably entered Hawaii as eggs glued on aircraft or on aircraft cargo. It is also possible that adult moths were brought in aircraft, but it is rather unlikely that larvae or pupae were so introduced.

Although no reference to a polyhedrosis virus disease of *S. mauritia* was found, Anantanarayanan & Ramakrishna Ayyar (1937) reported a disease in South India which they considered as probably caused by bacteria. They failed to mention any polyhedral bodies in armyworms killed by the disease, but the symptoms described by them somewhat resembled those of the polyhedrosis found in Hawaii. It seems probable that the virus was introduced into Hawaii with its host rather than that the host acquired a virus of another insect species in Hawaii. This assumption is based on the general knowledge that insect viruses are usually host specific, but occasionally the same virus may be cross-transmitted to related species (Steinhaus 1952, Tanada 1954). However, Smith & Xeros (1952, 1953) reported extensive cross-transmission of insect viruses even between unrelated insect species. Because there is a possibility that a latent virus was activated in some of these cases (Smith & Rivers 1956), their results need to be confirmed by further evidence.

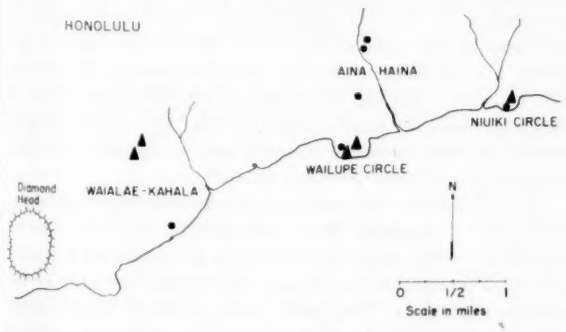

FIG. 1.—Portion of the city of Honolulu showing the localities where the virus-infected populations of the armyworm, *Spodoptera mauritia*, were observed. Black dots refer to localities with infected young armyworms in their first to fourth instars; triangles refer to those with infected armyworms in their fifth to seventh instars.

Laboratory attempts to transmit the polyhedrosis virus of *S. mauritia* to the cosmopolitan armyworm, *Pseudaletia unipuncta* (Haworth), and attempts to transmit the virus of the latter species to *S. mauritia* have all been unsuccessful. However, the host specificity of the virus of *S. mauritia* needs to be confirmed by testing with other insect species.

Insect viruses are known to be transmitted to susceptible hosts by many different methods. Steinhaus (1954) has discussed some of the various modes of transmission or dissemination of such pathogens. The virus may be transmitted: (1) on the body surface of mechanical carriers, such as healthy but susceptible insects or on scavengers and other nonsusceptible insects; (2) by insect parasites and predators; (3) by cannibalistic actions of the host insect; (4) by physical agencies such as wind, rain, irrigation water, and dust particles; and (5) within or on the surface of the egg (transovarial transmission).

It is rather unlikely that mechanical carriers and physical agencies were responsible for the initial distribution of the virus to *S. mauritia* in Hawaii. Such carriers would be expected to play a more important role in areas where the virus already is widely established, but would probably not act as the initial virus distributor within a recent immigrant species. Wind was probably of no importance in the original distribution of the virus, as the disease was found in areas upwind, with respect to the prevailing wind, from the site of the original discovery of the armyworm and over a part of the open sea. The role of insect parasites and predators appears also improbable for an initial distribution. Predators such as birds are known to distribute viruses through their fecal droppings, but here again they would be expected to play a more impotant part in areas with more extensive virus distribution. Cannibalistic action on the part of the host insects also was probably of negligible importance in dissemination, as this armyworm shows very little tendency towards cannibalism even when confined without food in small containers in the laboratory. Furthermore, cannibalism was not observed in any of the diseased armyworm populations in the field.

Dissemination by the adult females through the egg, either on the surface or within the egg, appears to be the most probable means of distribution of the polyhedrosis virus of *S. mauritia*. The previous assumption that the armyworm arrived in Hawaii in the egg stage would lend support to this conclusion. Moreover, the mode of distribution of the virus from the initial site of *S. mauritia* discovery to the localities where the virus-infected larvae were found (about 20 miles away) was most likely by females of succeeding generations through their virus-contaminated eggs. Inasmuch as over one-half of the virus-infected armyworm populations consisted of young larvae in their first to fourth instars, this would indicate that infection occurred at an early larval stage and possibly even in the egg. However, the small proportion of armyworm populations found infected and the absence of infected larvae in the windward areas of Oahu indicate either that transmission of the virus through the egg is infrequent or that some environmental factors may suppress the expression of the virus.

The assumed transovarial transmission of the polyhedrosis virus of *S. mauritia* may involve virus in the

latent or active state. If it were in the latent state, some mechanism in the environment or some inherent characteristic may activate the virus.

Another possible, although improbable, explanation for the rather sudden appearance of a virus disease in *S. mauritia* is the hypothesis of Yamafuji *et al.* (1947, 1951, 1951) that insect virus may be created from genes or gene-like material upon the action of certain chemicals or environmental factors. Thus far, this hypothesis has found very few followers, and Krieg (1955, 1956) has concluded from his tests that Yamafuji's results were an expression of latent virus infection.

SUMMARY.—A recently introduced armyworm, *Spodoptera mauritia*, was found infected by a polyhedrosis virus slightly more than 2 years after the armyworm was first found in Hawaii. The rather sudden appearance of a virus disease in this recent immigrant species has aroused some speculative discussion as to the origin and modes of distribution of the virus. It is believed that the virus entered Hawaii together with its host and very likely was disseminated by female moths through their eggs.

REFERENCES CITED

Anantanarayanan, K. P., and T. V. Ramakrishna Ayyar. 1937. Bionomics of the swarming caterpillar of paddy in South India. Agric. and Livestock in India **7**: 725–34.

Bianchi, F. Notes and exhibitions. Proc. Hawaiian Ent. Soc. (In press).

Krieg, A. 1955. Zur Frage einer "künstlichen Virus-Erzeugung" in *Bombyx mori* L. Naturwiss. **42**: 589–90.

Krieg, A. 1956. "Endogene Virusentstehung" und Latenzproblem bei Insektenviren. Arch. ges. Virusforsch. **6**: 472–81.

Pemberton, C. E. 1955. Notes and exhibitions. Proc. Hawaiian Ent. Soc. **15**: 373.

Smith, K. M., and C. F. Rivers. 1956. Some viruses affecting insects of economic importance. Parasitology **46**: 235–42.

Smith, K. M., and N. Xeros. 1952. Transmission of polyhedral viruses between different insect species. Nature **170**: 492.

Smith, K. M., and N. Xeros. 1953. Studies on the cross-transmission of polyhedral viruses: experiments with a new virus from *Pyrameis cardui*, the painted lady butterfly. Parasitology **43**: 178–85.

Steinhaus, E. A. 1952. The susceptibility of two species of *Colias* to the same virus. Jour. Econ. Ent. **45**: 897–9.

Steinhaus, E. A. 1954. The effects of disease on insect populations. Hilgardia **23**: 197–261.

Tanada, Y. 1954. A polyhedrosis virus of the imported cabbageworm and its relation to a polyhedrosis virus of the alfalfa caterpillar. Ann. Ent. Soc. America **47**: 553–74.

Tanada, Y. 1955. Notes and exhibitions. Proc. Hawaiian Ent. Soc. **15**: 384.

Yamafuji, K., and T. Cho. 1947. Weitere Studien zur Entstehung des Seidenraupenpolyedervirus ohne Virusinfektion. Biochem. Zeistschr. **318**: 95–100.

Yamafuji, K., H. Omura, and F. Yoshihara. 1951. Effect of chemicals on the virus infection in silkworms. Enzymologia **15**: 28–30.

Yamafuji, K., and I. Yoshihara. 1951. Procedures for the chemical production of silkworm virus. Enzymologia **15**: 10–13.

Laboratory Toxicity Tests Against Insects Affecting Sugar Beets Grown for Seed[1]

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More than half of the sugar-beet seed produced in the United States is grown in the Salt River Valley of Arizona. In this area the beets are planted in August and September and the seed is harvested in July and August of the following year. The beet leafhopper, *Circulifer tenellus* (Baker), as the vector of curly top, is one of the most importatnt pests of the crop in the fall, when the plants are small and most susceptible to the effects of the disease. From late April through May, when the seed is forming, it is usually necessary to treat most of the fields for the control of lygus bugs, principally *Lygus hesperus* Knight, which may seriously reduce the yield and viability of the seed crop. Experiments conducted on the relative toxicity of various common insecticides of the beet leafhopper and the lygus bug at Phoenix, Ariz., from 1954 to 1955 are reported herein.

METHODS.—In each test two small sugar-beet plants about 3 inches apart in a 6-inch pot were dusted with the test insecticide by a vacuum method similar to that described by Farrar *et al.* (1948). The plants and a weighed charge of dust were placed under the bell jar and the atmospheric pressure was reduced to 15 inches with a vacuum pump. Removal of a small stopper in the top of the bell jar allowed the air to pick up the dust from a small receptacle and distribute it evenly over the plants. Immediately after dusting one of the two plants was covered with a cloth-capped cylindrical cage made of cellulose acetate and infested with 10 test insects from the field. The plants were held in the greenhouse and mortality records made in 24 or 48 hours. Moribund insects were recorded as dead. After 1 week the second plant in each pot was similarly infested and examined. Replications of the tests were made on different days.

In tests of the systemic insecticides demeton, schradan, and Am. Cyanamid 3911 and 12008, 1% solutions or emulsions were added to the top of the soil with a pipette at rates comparable to 8 and 16 pounds per acre of the active materials. The pots were watered immediately after application to distribute the materials. Special cages made of cloth and cellulose acetate were used to confine leafhoppers to individual leaves that extended over the edges of the pots so that the insects would not be

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Table 1.—Percentages of mortality of adult beet leafhoppers caged for 48 hours on small sugar beets immediately after treatment with different insecticides, and 1 week after treatment.

TREATMENT	IMMEDIATELY AFTER DUSTING (11 REPLICATES)	1 WEEK AFTER DUSTING (10 REPLICATES)
Parathion 2%	97	13
Chlorthion 5%	88	20
Malathion 5%	72	19
Diazinon 4%	71	15
DDT 5%	57	21
Dieldrin 2%	50	28
Chlordane 5%	41	20
Endrin 1%	32	11
Toxaphene 15%	29	13
Heptachlor 2.5%	24	11
BHC (2% gamma)	24	13
Strobane 5%	24	12
Perthane 5%	18	14
Untreated	1	5
L.S.D. at 5% level	9.5	9.9

exposed to the treated soil or the volatilization of the chemicals. The toxicity of the leaves of the treated plants was tested every 5 or 10 days with a fresh lot of leafhoppers. For these tests 10 adult leafhoppers were put in each cage and held for 24 hours.

TESTS ON THE BEET LEAFHOPPER.—The results of some tests with insecticide dusts on the beet leafhopper are summarized in table 1. These data show that the four phosphorus compounds—parathion, malathion, Chlorthion, and Diazinon—were significantly more toxic immediately after application than any of the other materials at the strengths used. DDT and dieldrin ranked next and were significantly more toxic than chlordane, endrin, heptachlor, BHC, Strobane, or Perthane. One week after dusting all the dusts had lost much of their toxicity. At this time dieldrin was significantly more toxic than all the other materials except chlordane, DDT, malathion or Chlorthion, which were the only ones that showed any significant residual action.

The systemic insecticides demeton, schradan, Am. Cyanamid 3911, and 12008 showed a higher toxicity at the rate of 16 pounds than at 8 pounds per acre. Data for both rates of application were combined in table 2, where all four materials show considerable toxicity for more than 2 months, after which the tests were discontinued.

Table 2.—Percentages of mortality of adult beet leafhoppers caged on potted sugar beets for 48 hours at different intervals after systemic insecticides were added to the soil.

DAYS AFTER TREATMENT	DEMETON	SCHRADAN	AM. CYANAMID 3911	AM. CYANAMID 12008	UNTREATED CHECK
1	100	74	98	98	28
5	91	42	98	95	16
10	78	43	98	100	7
15	93	67	100	98	5
20	88	62	100	98	13
25	71	38	100	98	12
30	58	65	95	100	0
35	51	53	98	97	17
40	70	58	100	100	0
45	40	35	100	100	0
50	56	42	100	98	10
60	70	30	90	85	0
70	30	32	70	98	0
Average	75±8	54±3	97±1	98±1	6±1

Demeton was significantly more toxic than schradan, and 3911 and 12008 were significantly more toxic than demeton.

TESTS ON LYGUS BUGS.—The results of some tests on lygus bugs based on 11 replicates were as follows:

Treatment	*Per Cent Mortality*
Dieldrin 2%	100
Chlorthion 5%	98
Heptachlor 2.5%	97
Malathion 5%	95
Parathion 2%	93
Diazinon 4%	85
Endrin 2%	84
Toxaphene 10%	82
Strobane 5%	81
DDT 5%	79
Perthane 5%	52
Untreated	25
L.S.D. at 5%	11

It will be seen that many of the materials were more toxic than DDT and the related compounds Perthane and Strobane. The phosphorus compounds Chlorthion, malathion, parathion, and Diazinon were relatively high in toxicity as were also dieldrin and heptachlor. Endrin and toxaphene were intermediate.

A second series of tests on lygus bugs was made to obtain more data on immediate toxicity and also some information on the residual toxicity of the chemicals. As shown in table 3, all the insecticides seemed to have considerable toxicity. As in the previous tests, the phosphorus compounds ranked high in toxicity. Immediately after treatment malathion was more toxic than any of the other materials and parathion was better than any of the remaining compounds except endrin and dieldrin. One week after application all the materials except Am. Cyanamid 3911 and 12008 had lost most of their toxicity, whereas these two materials had increased in toxicity due apparently to systemic action. Later observations showed that the strong residual effect of 3911 and 12008 continued for at least 35 days. Of the different dusts only

Table 3.—Percentages of mortality of adult lygus bugs caged for 24 hours on small sugar beet plants immediately after treatment with different insecticides, and 1 week after treatment.

TREATMENT	IMMEDIATELY AFTER TREATMENT	1 WEEK AFTER TREATMENT
Dusts:		
Malathion 5%	78	13
Parathion 2%	62	31
Endrin 1.5%	52	8
Dieldrin 2%	49	10
BHC (2% gamma)	48	8
DDT 5%	41	13
Toxaphene 15%	39	10
Heptachlor 2.5%	36	13
Chlordane 5%	36	9
Systemics applied to soil:[a]		
Am. Cyanamid 3911	51	90
Am. Cyanamid 12008	38	76
Untreated	11	5
L.S.D. at 5% level	14	12

[a] Applied at the rate of 4 pounds per acre.

parathion showed a significant residual effect after 1 week.

In other tests liquid applications, Am. Cyanamid 3911 and 12008 at 16 pounds per acre resulted in some phytotoxicity. When sugar-beet seeds were coated with special preparations of these materials in activated carbon, there was considerable stunting and curling of the leaves of the young plants, but the symptoms tended to disappear as the plants grew larger. When the seeds were soaked for 30 minutes in 1% solutions of demeton or schradan, or emulsions of 3911 or 12008, there were no adverse effects on germination, and the plants showed no chemical injury.

SUMMARY.—In greenhouse tests in Arizona in 1954 and 1955, the phosphorus compounds parathion, malathion, Chlorthion, and Diazinon were more toxic to the beet leafhopper, *Circulifer tenellus* (Baker), than nine other common insecticides. In similar tests the phosphorous materials were highly toxic to adults of the lygus bug, *Lygus hesperus* Knight. DDT was more toxic to the leafhopper than to the lygus bug. On the other hand, dieldrin, endrin, and heptachlor seemed to be more toxic to lygus bugs. Much of the toxicity of the dusts was lost by the end of a week. At this time chlordane, dieldrin, DDT, malathion, and Chlorthion showed significant residual action on the leafhoppers, but only parathion showed any significant residual effect on the lygus bugs.

When systemic insecticides were applied to the soil around potted plants, a high toxicity to the leafhopper extended over 2 months, after which the tests were discontinued. Demeton was more toxic than schradan, and Am. Cyanamid 3911 and 12008 were more toxic than demeton. Similar soil treatments with 3911 and 12008 were toxic to the lygus bugs for at least 35 days. Seed treatments with these two materials made the plants toxic to both insects for considerable periods.

REFERENCE CITED

Farrar, M. D., W. C. O'Kane, and Howard Smith. 1948. Vacuum dusting of insects and plants. Jour. Econ. Ent. **41**(4): 647–8.

Oviposition Sites of the Pink Bollworm on the Cotton Plant[1]

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The investigation reported herein was designed to determine the preferred site of oviposition by the pink bollworm, *Pectinophora gossypiella* (Saund.), on the cotton plant. Plants ranging in age from the 4-leaf stage to maturity were included in the studies. Previous reports on the oviposition habits of this insect were confined to studies of the distribution of eggs on the cotton plant when bolls were present.

Busck (1917) reported that most pink bollworm eggs were laid near the apex of the green boll in the longitudinal depressions. Loftin (1921) found 51.7% of the eggs on the bolls, 0.8% on the squares and the remainder scattered over the vegetative parts of the plant. He also found that 30.5% of the eggs on the bolls were located at the base of the bolls. Ohlendorf (1926) reported that the favorite place for oviposition was under the calyxes of bolls. Hunter (1926) found eggs on all parts of the plant above ground with about 50% on green bolls. The preferred oviposition site on green bolls was under the calyx. In a 3-year study, Fenton & Owen (1953) found 88.4% of the eggs inside the involucre of the bolls and none on squares. They stated that during the early part of the season before boll development, eggs were deposited indiscriminately over the cotton plant.

Usually the peak of emergence of overwintered pink bollworms occurs before bolls are present on cotton. Therefore practically all of the second generation pink bollworms that attack bolls are probably the progeny of an earlier generation that developed on cotton squares. This early generation must have developed from eggs laid on the cotton plant by adults of the overwintered pink bollworms before bolls were present. Therefore a knowledge of the activity and habits of the overwintered generation of pink bollworms may be important in the planning of control measures. The place of oviposition is particularly important since the location of the eggs on the plant may influence the effectiveness of chemicals applied for control of larvae or the efficiency of predators and parasites in destroying eggs and larvae.

EXPERIMENTAL PROCEDURES.—*Method of obtaining moths.*—Pink bollworm moths that had emerged from infested seed cotton collected during the previous season were used to infest the cotton plants. The infested seed cotton was harvested during November, 1954 and held during the winter at 40° F. Infested seed cotton was taken from refrigeration as moths were needed and placed under a pyramid-type screen emergence cage measuring 3 feet square at the base and 2 feet high. A 1-pint glass collecting jar with a mouth 2 inches in diameter was inverted and screwed into a holder at the top of the cage. A screen cone with a 5-millimeter hole at the top center was placed in the mouth of each collecting jar which permitted moths to enter the jar, but prevented escape.

The emergence cages were located in a shady area outside the laboratory. The infested seed cotton was spread in a layer underneath the cage and moistened by sprinkling with water at weekly intervals. The glass collecting jars were inspected daily and the moths removed. Moths were immobilized with carbon dioxide and lots of 10 each were placed in glass vials for use in the tests.

Testing procedure.—Deltapine 15 cotton planted in

[1] The studies reported herein were conducted jointly by the Louisiana and Texas Agricultural Experiment Stations at the Entomology Research Laboratory, Texas Agricultural Experiment Station, College Station in cooperation with the Entomology Research Branch, U. S. Department of Agriculture. Accepted for publication August 28, 1956.

[2] Louisiana Agricultural Experiment Station, Baton Rouge.
[3] Texas Agricultural Experiment Station, College Station.

rows in the field was used in this test. The pink bollworm moths were confined on plants in 2×2×2 feet screen cages. One cotton plant was covered by each cage except in the first two tests in which three plants were used because of the extremely small size of the plants.

Cages used to confine pink bollworm moths on the cotton plant were of a wooden frame construction covered with two layers of screen. The outer layer of screen was regular 14×18 mesh bronze wire and the inner layer was 32×32 mesh plastic screen. One entire side of the cage was removable, fitting into grooves at the bottom and fastening with turnbuttons at the top. The bottom of the cage was removable and consisted of two pieces of $\frac{3}{8}$-inch plywood fitted together with a hole in the center to receive the stem of the cotton plant. These bottom pieces rested on a base with 8-inch legs.

The cages were installed over the cotton plant by leveling the cage base around the plant and placing the two bottom pieces in position with the stem of the cotton plant through the center hole. The space between the plant stem and cage bottom was sealed with caulking compound. Masking tape was used to seal the crack between the two bottom pieces. The cage was placed on the base over the plant and masking tape used to seal the cracks between the cage and bottom boards.

After the cages were installed over the cotton plants, 10 moths were introduced into each cage except in the first test in which 15 moths were used per cage. Five days after moth release the plants were removed from the cages and examined under a binocular microscope for number and location of eggs. The vegetative parts examined included leaves, terminals and axils of limbs and leaves. The examination for eggs on the fruiting parts included on the boll inside the bracts, under the boll calyx, around the nectaries of bolls and squares, and inside the involucre of the squares. The cotton was in the 4-leaf stage during the first test. Weekly tests were conducted thereafter until the bolls began to open.

RESULTS.—The percentages of pink bollworm eggs found at various locations on the cotton plant at different stages of development are given in table 1. The terminals were the favorite vegetative part used as sites for oviposition by the moths. Until considerable numbers of bolls became available about July 13, over 50% of the total number of eggs deposited were found in terminals. The eggs were found in the heavy pubescence on young leaves and on unfolding leaves.

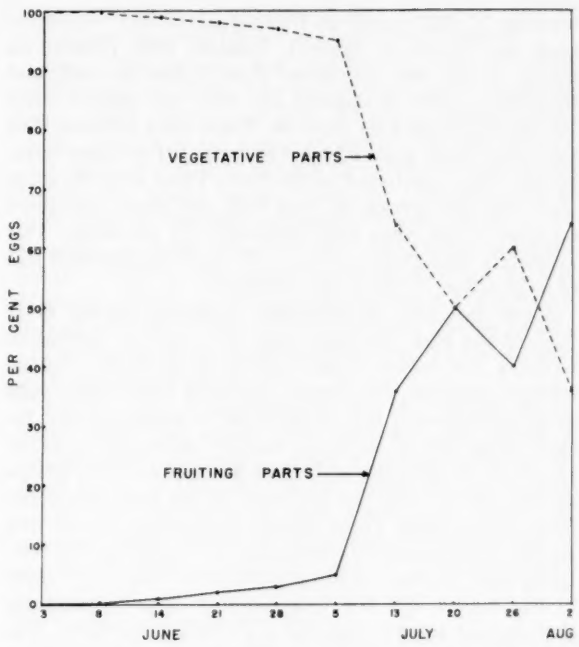

FIG. 1.—Distribution of pink bollworm eggs found on the cotton plant at College Station, Texas. First squares found in the test of June 8. First bolls of at least 10 days of age found in test of June 28.

The next vegetative site in order of preference for oviposition was the axils of leaves and limbs. Eggs were placed on the fuzzy axillary buds, in the angle formed by the axillary buds with stems and petioles, and under the stipules. The percentages of eggs found in this location were relatively constant throughout the experiment.

Leaves were the least preferred of the vegetative parts for oviposition. Eggs found on leaves were invariably located in the acute angles formed where the leaf veins radiate out from the junction of the leaf blade and petiole.

A total of five eggs was found inside the involucre of two squares during the course of the study. Low percentages of eggs were found around the nectaries of bolls and squares. It was surprising that the squares were not preferred for oviposition, particularly before bolls were available, since the elongate tips of the square calyx appeared to offer an ideal site for oviposition. The relative

Table 1.—The influence of the stage of development of the cotton plant on the site selected for oviposition by the pink bollworm moth.

Date of Test	Replications	Per Cent Eggs on Vegetative Parts				Per Cent Eggs on Fruiting Parts					Mean No. Bolls per Plant	Total Eggs	Eggs per Moth
		Leaves	Terminals	Axils	Total	Squares	Under Calyx	On Boll	Fruit Nectaries	Total			
June 3	20	5.3	61.2	33.5	100.0	0.0	0.0	0.0	0.0	0.0	0.0	2996	10.0
8	10	3.6	54.3	42.1	100.0	0.0	0.0	0.0	0.0	0.0	0.0	1018	10.2
14	10	6.3	78.0	14.6	98.9	0.0	0.0	0.0	1.1	1.1	0.0	707	7.1
21	10	7.0	78.7	12.4	98.1	0.0	0.0	0.0	1.9	1.9	0.0	1306	13.1
28	10	5.2	68.3	23.7	97.2	0.0[a]	1.0	0.0	1.8	2.8	0.3	3203	32.0
July 5	9	9.7	55.5	29.6	94.8	0.0	1.4	0.0	3.8	5.2	0.6	3471	38.6
13	10	11.0	31.3	21.7	64.0	0.0[b]	22.5	11.4	2.1	36.0	3.8	3047	30.5
20	5	6.4	29.1	14.5	50.0	0.0	22.5	26.2	1.3	50.0	4.4	2080	41.6
26	7	17.3	27.0	15.7	60.0	0.0	23.2	13.4	3.4	40.0	8.9	3707	53.0
August 2	7	8.5	18.2	9.3	36.0	0.0	45.9	9.2	8.9	64.0	12.0	4183	59.8

[a] Two eggs of 3203 found on one square.
[b] Three eggs of 3047 found on one square.

number of eggs found on fruiting parts and vegetative parts are shown in figure 1. Squares were present on plants in the test conducted June 8 and in each test thereafter. Bolls 10 days of age were not present until the test conducted on June 28. These data indicate that the oldest bolls were 2 to 3 weeks old before large numbers of eggs were found on the bolls. From July 13, when there was an average of four bolls per plant, one-third or more of the eggs were deposited on the bolls. This indicated that green cotton bolls were the favored site of oviposition of the pink bollworm.

These data may be significant in another regard. Over 90% of the pink bollworm eggs were laid on vegetative parts of the cotton plant until 4 weeks before cotton started opening. The larvae hatching from these eggs must migrate over the cotton plant in search of fruit for food. Thus natural mortality may be higher and exposure to predators, parasites and chemicals applied to the plants greater than for larvae hatching in the more protected sites on the bolls. These adverse conditions for the first and part of the second generation could explain the relatively slow increase of the population of this insect early in the season and the tremendously large populations that develop later in the year after bolls are present. The proportion of eggs found on the fruit was not greater than on vegetative parts until the test on August 2 when an average of over 12 bolls per plant was present.

According to the data on average number of eggs laid per moth caged on the plants shown in table 1, the rate of egg-laying of the pink bollworm moths appeared to increase as the season advanced. This was surprising in view of the fact that all moths were obtained in the same manner and from overwintered larvae of the previous season. It was not known if these increasing numbers of eggs found per moth indicated an increased reproduction rate as the season advanced or, that in the absence of the preferred oviposition sites on green cotton bolls, many eggs were laid in cracks and other locations off the plant. The latter possibility seemed the more likely since Fenton & Owen (1953) found pink bollworm eggs in the soil.

SUMMARY.—A study was conducted at College Station, Texas to determine the relative numbers of pink bollworm eggs deposited on vegetative and fruiting parts of the developing cotton plant. Over 90% of the eggs were found on vegetative parts of the plant for the first 6 weeks of plant development. During the next 4 weeks, as green cotton bolls became more numerous on the plants, an increase in the number of eggs on bolls and a decrease on vegetative parts was noted. The percentage of eggs on fruiting parts did not exceed those on vegetative parts until the final test on August 2 when bolls were beginning to open.

REFERENCES CITED

Busck, A. 1917. The pink bollworm, *Pectinophora gossypiella*. Jour. Agric. Res. 9: 343–70.

Fenton, F. A., and W. L. Owen. 1953. The pink bollworm of cotton in Texas. Texas Agric. Expt. Sta. Misc. Publ. 100.

Hunter, W. D. 1926. The pink bollworm, with special reference to steps taken by the Department of Agriculture to prevent its establishment in the United States. U. S. Dept. Agric. Bull. 1397.

Loftin, U. C., K. B. McKinney and W. K. Hanson. 1921. Report on investigations of the pink bollworm of cotton in Mexico. U. S. Dept. Agric. Bull. 918.

Ohlendorf, W. 1926. Studies of the pink bollworm in Mexico. U. S. Dept. Agric. Bull. 1374.

Control of the Spotted Alfalfa Aphid on Alfalfa[1]

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During January, 1955, the spotted alfalfa aphid, *Pterocallidium* sp.,[2] was found in destructive numbers on alfalfa in Brazos County, Texas. Only about a year earlier or during February, 1954, this species was first recorded from the United States in central New Mexico by Dickson *et al.* (1955). This insect was known at that time as the yellow clover aphid. The spotted alfalfa aphid also attacks bur clover (*Medicago hispida*) in Texas. In addition to alfalfa and bur clover, Dickson & Reynolds (1955) have recorded this aphid feeding upon sour clover (*Melilotus indica*), black medic (*Medicago lupulina*), and to some extent berseem (*Trifolium alexandrinum*). Dickson *et al.* (1955) report that this aphid was first collected in California from bur clover in February, 1954, by R. F. Wilkey. Damaging infestations of the spotted alfalfa aphid on alfalfa in Arizona were reported in May and June, 1954, by Tuttle & Butler (1954).

Spotted alfalfa aphids damage the plants by sucking the juices from the leaves and tender stems. They may inject a toxin that interferes with the growth of the plant. The leaves curl, turn yellow and drop off. Shedding of the lower leaves usually occurs first. Severely damaged plants are almost defoliated. Young and old alfalfa stands are greatly reduced and in some cases almost destroyed. These insects secrete honeydew which is a medium for the growth of sooty molds. Alfalfa with heavy deposits of honey dew and sooty molds cannot be dehydrated properly, and it is difficult to cut and bale.

EXPERIMENTAL PROCEDURE.—All treatments and checks were randomized and replicated four times. One-tenth acre plots were used. Insecticide sprays were applied with a self-propelled machine operated at approximately 4 miles per hour. Five to 6 gallons of spray mixture were delivered through No. 3 cone nozzles at a pressure of 60 pounds per square inch. Granulated materials used in 1956 were applied with a cyclone hand seeder.

Sampling of aphid populations during 1955 consisted of the removal of 10 clumps of alfalfa at random from each plot. Clumps of alfalfa plants, taken with a 5-inch

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[2] Identified by L. M. Russell, U. S. Dept. of Agriculture.

Table 1.—Results of sprays applied to alfalfa at College Station, Texas, for control of the spotted alfalfa aphid. 1955.

Material[a]	Pounds of Toxicant per Acre	Initial Infestation Aphids per 10 Clumps, Feb. 1	Average Infestation and Per Cent Control			
			Feb. 2		Feb. 9	
			Average	Per Cent Control	Average	Per Cent Control
Malathion	0.33	134.0	30.3	83.9	3.3	97.7
Parathion	0.25	99.0	10.5	92.4	3.3	96.9
BHC (g.i.)	0.4	151.5	23.8	88.8	3.5	97.9
Toxaphene-DDT (2-1)	3.0	95.0	58.5	56.0	13.3	86.9
DDT	1.5	141.5	95.8	51.6	11.5	92.4
Check	None	82.0	114.8		87.8	

[a] Applied February 1, 1955.

pointing trowel, were placed in paper bags and the soil and plant material were washed and screened to separate the aphids for counting. Infestation records were taken during 1956 by bending and pinching a 3-inch terminal of an alfalfa stem so that the sample portion fell into an ice-cream container. Extreme caution was taken to prevent the aphids from falling to the ground as they do when disturbed. Ten samples were taken at random from each plot. The contents in each carton were fumigated with carbon tetrachloride and the aphids counted.

RESULTS.—Infestations of the spotted alfalfa aphid were considerably less in 1955 than during 1956 (tables 1, 2 and 3). Although the methods of taking infestation records were different during the 2 years, the plant clump technique used in 1956 showed higher counts of aphids than the terminal sample method used during the same period. Infestations of aphids were more localized during 1955 than 1956. Consequently less migration occurred which may account for the good control obtained.

Heavy infestations and migrations of aphids during 1956 were more difficult to control than the lower populations which occurred in 1955. In most instances malathion, at 0.33 to 1.0 pound of active toxicant per acre, and parathion at 0.25 pound, gave fair to good control for about 2 weeks (tables 1 and 2). Demeton also was fairly effective for approximately 2 weeks (table 2). Unfavorable growing conditions may have been responsible for the relatively short period of effectiveness of demeton. High initial kills were obtained with endrin spray (table 3) and granules of Am. Cyanamid 3911 (tables 2 and 3) but neither had a lasting effect. No control was obtained with granulated endrin.

The presence of large populations of beneficial insects, particularly lady beetles and lacewings, apparently accounted for reductions in aphid populations during mid-April (table 3). Beneficial insect populations were greatly reduced in plots treated with granulated endrin since aphid infestations actually increased a few days after the application of the insecticide (table 3). High populations of beneficial insects occurred in these plots about 1 week later than in other treated plots.

SUMMARY.—A new pest in Texas, the spotted alfalfa aphid, was found damaging alfalfa near College Station in January, 1955. It has become increasingly injurious to alfalfa. The small, active aphids cause injury by sucking juices from the leaves and stems and by secreting honeydew in which sooty molds grow.

Three control tests, including several insecticides, were

Table 2.—Insecticide tests for control of the spotted alfalfa aphid on alfalfa at College Station. 1956.

Material[a]	Pounds of Toxicant per Acre	Initial Infestation Aphids per 10 Terminals, March 6, 56	Average Infestation and Per Cent Control					
			March 8		March 17		March 20	
			Average	Per Cent Control	Average	Per Cent Control	Average	Per Cent Control
Demeton	0.25	651.0	124.5	72.3	139.9	84.9	633.5	48.8
Am. Cyanamid 3911 (granules)	.65	681.8	150.5	67.9	98.8	89.8	1006.0	22.4
Toxaphene-DDT (2-1)	3.0	661.3	50.5	89.0	18.3	98.0	305.8	75.7
Malathion	.75	582.2	48.0	88.1	238.3	71.2	859.7	22.3
Parathion	.25	711.8	56.3	88.5	118.8	88.2	1081.3	20.1
Check	None	551.8	380.3		784.3		1049.1	

[a] Applied March 6 except Am. Cyanamid was applied in the early morning of March 8.

Table 3.—Insecticide tests for control of the spotted alfalfa aphid on alfalfa at College Station. 1956.

Material[a]	Pounds of Toxicant per Acre	Initial Infestation Aphids per 10 Terminals, March 24, 56	Average Infestation and Per Cent Control									
			March 26		March 30		April 3		April 13		April 19	
			Average	Per Cent Control	Average	Per Cent Control	Average	Per Cent Control	Average	Per Cent Control	Average	Per Cent Control
Endrin	0.3[b]	570.5	34.3	93.7	329.7	52.1	138.8	0.0	220.5	0.0	358.5	0.0
Am. Cyanamid 3911 (granules)	0.67	861.7	36.3	93.3	324.5	52.8	90.8	0.0	25.8	75.4	30.3	45.0
DDT	1.0	741.2	92.8	82.9	288.2	58.1	83.3	0.0	19.8	79.7	28.3	44.0
Malathion	0.5[c]	676.7	62.8	88.4	397.5	42.2	105.5	0.0	30.8	72.7	38.8	39.4
Toxaphene-DDT (2-1)	3.0	746.7	88.8	83.6	334.2	51.4	100.5	0.0	6.8	94.3	19.0	68.9
Check	None	805.5	543.0		688.0		82.0		199.5		49.8	

[a] Applied March 24 and April 7, except as indicated below.
[b] Granulated endrin at 0.25 pound per acre applied on April 7, 1956.
[c] Malathion spray at 1.0 pound per acre applied April 7, 1956.

conducted during 1955 and 1956. Samples of alfalfa clumps and stem terminals were used for obtaining infestation records. Of the insecticides tested, malathion at 0.5 and 1.0 pound of active material, parathion at 0.25 pound of active material and toxaphene-DDT at 3.0 pounds of active material per acre were the most effective insecticides used for combatting the aphid. Fairly good control was obtained with these materials for only 1 to 2 weeks after treatment.

REFERENCES CITED

Dickson, R. C., Edward F. Laird, Jr., and George R. Pesho. 1955. The spotted alfalfa aphid (yellow clover aphid on alfalfa). Hilgardia **24**(5): 93–118.

Dickson, R. C., and H. T. Reynolds. 1955. The yellow clover aphid on alfalfa. California Agric. **9**(7): 4, 15.

Tuttle, D. M., and G. D. Butler, Jr. 1954. The yellow clover aphid—a new pest in the southwest. Jour. Econ. Ent. **47**(6): 1157.

On the Biology and Egg-Laying Habits of the Western Corn Rootworm[1]

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The western corn rootworm[2] was first recognized in 1909 as a pest on corn in Colorado (Gillette 1912). During a 1948 survey by Hixson *et al.* (1949) the beetle was prevalent as far east as Seaward and Saline counties in Nebraska, approximately 70 miles from the Missouri River. In a survey conducted by the author in 1954, the beetle was collected all along the Missouri River from South Dakota to Missouri. This rapid easterly migration of the beetle during recent years cannot be adequately explained.

The western corn rootworm together with the southern[3] and northern[4] species constitute the major economic insect pests of corn, second in importance only to the European corn borer. The loss caused by the various rootworms is hard to ascertain, but it probably equals that resulting from corn borer feeding. Although considerable information is available regarding control of the several species of rootworms, including the western species, it was felt that more attention should be given to learning more about some of the habits of the western species. This paper is specifically concerned with the habits of the western corn rootworm as observed during the 1953, 1954 and 1955 seasons in the Lincoln, Nebraska area.

LIFE CYCLE—*Field Studies.*—In contrast to some of the other Chrysomelidae, the western corn rootworm overwinters in the egg stage. The eggs are laid in the ground near the base of the corn plant in the middle to late summer. In order to determine the depth at which the eggs are laid, a series of cages 3 feet square and 8 feet high was buried in the soil in the early spring, in such a manner that the screened bottoms rested 2, 4, or 6 inches from the soil surface. The soil removed to place the cage in position was replaced inside the cage. Corn was planted inside the cages and the adult beetles were counted daily during the emergence period later in the summer. The corn field containing the cages was typical of the area and supported an average population of corn rootworm beetles the previous year. Based on the number of adults that emerged from the various cages, it appears that approximately 23% of the eggs were laid in the upper 2 inches of soil, 58% in the upper 4 inches, and 80% in the upper 6 inches. The remaining 20% of eggs were probably laid below the 6-inch level.

In the Lincoln area adults of the western corn rootworm first appear in the field very early in July. Early feeding by the adult beetles occurs on the corn leaf surface. Later, as the silks and tassels appear, the beetles move to these areas for the major part of their feeding. As the season progresses, the beetles congregate in clumps of volunteer corn when available, appearing to prefer the younger, more succulent plants or the cooler, more sheltered habitat in which to feed. With the drying of the silks and tassels the husk is invaded and the kernels on the tip of the ear are hollowed out and eaten. At this time a comparatively small number of adult beetles move to nearby alfalfa and kochia, *Kochia scoparia* (L.), where they feed to a limited extent on the respective blossoms. In the central parts of the state Hixson *et al.* (1947) reported the beetle abundant in alfalfa fields and also in pastures and stubble fields where they fed on various weeds.

Although most of the mating in the field occurs throughout July and into August, some mating was observed as late as October. Virgin insects caught upon emergence from the soil and placed in vials were noted to mate shortly following capture At other times mating was not observed up to 6 days following emergence The required length of time *in copula* for successful mating was not determined. However, one pair of western rootworms remained in the mating position for almost 3 hours. In many instances, matings or attempted matings were observed on more than one occasion for a given pair of insects. In the field it is not uncommon to notice an adult western corn rootworm assume the mating position with a member of the northern species. The most frequent pairing consists of a northern male and a western female. Eggs were obtained from such a mating by Burkholder (1956) but a subsequent hatch was unobtainable.

Egg laying usually commences during the second or third week of August. In general, egg laying continues intermittently up to the time of killing frost. Eggs laid in the late summer and fall overwinter in the soil and do not hatch until the following spring. However, diapause can be broken artificially by chilling the eggs for prescribed periods at various temperatures (Spackman 1954).

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[2] *Diabrotica virgifera* Lec. (Coleoptera, Chrysomelidae).

[3] *Diabrotica undecimpunctata howardi* Barber.

[4] *Diabrotica longicornis* (Say).

The longevity of the individuals varied from 16 to 84 days. The average age for the adult female rootworm was 56.6 days in 1954 and 47.2 days in 1955. Records for male longevity recorded in 1954 indicated that the average age on death was 44.7 days, or approximately 25% shorter than that of the female.

OVIPOSITION RECORDS.—*Insectary Studies.*—Oviposition records for 20 caged females were obtained throughout the 1954 and 1955 seasons. The pairs of insects were caged in 1-×3-inch plastic vials containing a smaller ¼-×2-inch vial which held food and water. The food, which consisted of corn silk and anthers packaged in a small bundle, was placed in the smaller vial. The bottom of the 1-×3-inch vial was covered with black cheesecloth and inserted into a 3-inch square wooden base drilled to receive the vial. The cages were than placed on moist blotting paper in the insectary. The cheesecloth vial bottoms and the blotting paper were inspected daily and the eggs were removed and counted. A recording thermograph was set up near the cages in order to obtain a constant recording of the ambient temperature in the area.

Table 1.—**Egg production, number of eggs laid, average number of eggs laid per day and longevity of female western corn rootworm beetles. 1954, 1955.**

FEMALE	TOTAL NUMBER DAYS EGGS LAID	TOTAL EGGS LAID	AVERAGE NUMBER EGGS LAID PER LAYING DAY	AGE IN DAYS ON DEATH
		1954		
A	6	392	65.3	38
B	—	—	—	39
C	—	—	—	40
D	—	—	—	41
E	8	502	62.7	81
F	7	488	69.7	48
G	5	404	80.8	48
H	4	247	61.7	37
I	7	420	60.0	86
J	5	322	64.4	73
K	4	258	64.5	46
L	—	—	—	24
M	4	189	47.2	59
N	4	189	47.2	59
O	9	513	57.0	86
P	6	314	52.3	86
Q	3	231	77.0	28
R	8	725	90.6	86
S	5	346	69.2	85
T	4	236	59.0	72
Mean	4.5	288.8	51.4	56.6
		1955		
A	14	698	49.8	52
B	13	806	62.0	75
C	5	201	40.2	26
D	5	152	30.4	55
E	11	531	48.2	35
F	5	283	56.6	23
G	5	210	42.0	35
H	12	585	48.7	88
I	13	388	29.8	47
J	3	105	35.0	84
K	—	—	—	9
L	15	1045	69.6	54
M	8	266	33.2	54
N	5	136	27.2	84
O	11	574	52.1	26
P	12	796	66.2	84
Q	—	—	—	35
R	4	86	21.5	16
S	14	249	17.7	49
T	—	—	—	21
Mean	7.8	355.5	36.5	47.2

In 1954 egg laying started during the third week of August, whereas in 1955 the onset of oviposition was 1 week earlier. A summary of various information derived from the oviposition records is presented in table 1. The data in table 1 indicate that in 1955 the adult females oviposited almost twice as frequently on the average as in 1954. However, the average number of eggs laid per female during the 1955 season was only 46 more than during 1954. The average number of eggs laid per female on days eggs were laid was greater in 1954 than 1955. This is no doubt due to the fact that eggs were laid on fewer days in 1954. In 1954 the greatest number of eggs (475) was laid on August 27 and in 1955 the largest daily ovipositional record (705) was recorded on August 25. The number of females which did not lay varied from 15% in 1955 to 25% in 1954.

A comparison of the thermographic record with the record of egg deposition revealed the following information. In 1954 eggs were not oviposited when the evening temperature, or the minimum for the day, was below 50° F. In 1955 only in three instances were eggs laid when the minimum temperature was below 50° F. Optimum evening temperatures for egg laying appears to range between 60° and 65° F. The period of greatest oviposition during both years extended from August 20 to September 20, with a slack period during the first week of September when the mean temperature was slightly lower than for the period as a whole. A general relation between higher mean temperatures and increased egg deposition was in evidence.

SUMMARY.—The western corn rootworm, *Diabrotica virgifera* Lec. has recently finished migrating across Nebraska to the Missouri River.

Eighty per cent of the eggs of the western corn rootworm are laid in the upper 6 inches of soil, 58% in the upper 4 inches and 23% in the upper 2 inches. The remaining 20% are probably laid below the 6-inch level.

Adults appeared in the field from early July to October in the Lincoln, Nebraska area. Mating occurred shortly following emergence and egg laying commenced in early to mid-August and continued as long as the evening temperature was above 50° F. Longevity varied from 16 to 84 days.

A general relation existed between higher mean temperatures and increased oviposition. The average number of eggs laid per female during the season varied from 372 in 1954 to 418 in 1955.

REFERENCES CITED

Burkholder, W. E. 1956. Influence of food plants on fecundity of the corn rootworms, *Diabrotica virgifera* Leconte and *D. longicornis* (Say). (Coleoptera, Chrysomelidae). Unpublished M. S. Thesis, Univ. of Nebraska.

Gillette, C. P. 1912. *Diabrotica virgifera* Leconte as a corn rootworm. Jour. Econ. Ent. 54(4): 364–6.

Hixson, E., R. E. Hill, and M. H. Muma. 1947. 60th Annual Report, Agric. Experiment Sta., Univ. Nebraska, p. 52.

Hixson, E., M. H. Muma and R. E. Hill. 1949. 62nd Annual Report, Agric. Experiment Sta., Univ. Nebraska, p 67.

Spackman, E. W. 1954. The effect of temperature and chemical treatment on breaking the diapause in the eggs of corn rootworms, *Diabrotica longicornis* (Say) and *D. virgifera* Leconte. (Coleoptera, Chrysomelidae). Unpublished M. S. Thesis, Univ. of Nebraska.

Experiments for the Control of Spider Mites on Lima Beans[1]

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Four species of spider mites attack lima beans in southern California. The two-spotted spider mite, *Tetranychus telarius* (L.), has been an important pest since 1941, and *T. deviatarsus* McG. also damages this crop. *T. cinnabarinus* (Bois.) and *atlanticus* McG. have been found on lima beans in this area but are not of economic importance at present. Dusting sulfur was used as recommended by Elmore (1944) until about 1951, when it became evident that it was not giving adequate control. Experiments to find more effective materials were therefore carried on from 1952 to 1955.

METHODS.—The tests were conducted in fields of large lima beans grown for dry bean or seed production. These beans are usually seeded from May 1 to 15, cut from September 1 to 15, and threshed in September or October. Small plots were 1 to 8 rows wide and 30 to 50 feet long, with 4 to 8 replicates in randomized-block experiments. In the small plots dusts were applied with rotary hand dusters at 20 to 30 pounds per acre and sprays with a high-pressure mist sprayer at 5 to 10 gallons per acre. Large plots varied from 70 to 2300 feet long and from 8 to 14 rows wide, with 5 to 8 replicates, usually in randomized blocks, but in one test the block design was systematic. Applications in the large plots were made with a power duster that had booms long enough to treat 8 or 14 rows at one time, or with a power sprayer equipped to treat 8 rows at one time with 2 nozzles per row.

Mite counts on 10 leaflets, or half-leaflets, per plot were made at weekly or 2-week intervals following application. Yields were taken by cutting and threshing 100 feet of row per plot.

COMPARISON OF DIFFERENT INSECTICIDES (TABLE 1). —*1952 Experiment.*—Twelve materials were tested on small plots. Applications were made on June 11 and July 9, and the mite counts between June 9 and August 8.

Good control of the two-spotted spider mite was obtained with demeton, Aramite, and Allied Chemical's 876 (20%), fair control with NPD, ovex, and malathion, and poor control with parathion, DN-289, Allied Chemical's 876 (10%), Chlorobenzilate, sulfur, and Metacide. Mite populations were too low to show differences in crop yield between treatments, but all yields were higher than in the untreated plots in an adjoining experiment.

In 1952 tests were also made comparing demeton sprays and dusts, in which it was found that sprays were more effective than dusts (Wilcox & Howland 1954).

1953 Experiment.—Applications were made on small plots on June 11 and July 2, and the mite counts between June 16 and August 25.

Mite populations were low, and no significant differences in yield were apparent between treated and untreated plots. However, in reduction of mite populations all materials were significantly better than 100% sulfur, but there was no difference between the remaining treatments, or between treated and untreated plots.

1954 Experiment.—Applications were made on small plots on June 3 and 24, and mite counts were made between June 10 and July 29. No yield records were taken because of low mite populations.

All materials except sulfur were superior to the check. Demeton and Rohm & Haas FW-152 gave excellent control and were superior to malathion and to the materials less effective than malathion. Aramite and Rohm & Haas FW-293 gave good control and were superior to parathion and to the materials less effective than parathion.

1955 Experiments.—Four experiments were made in 1955. Experiment 1 was conducted on small plots after the plants had become heavily infested with mites. In the previous years applications were made prior to heavy mite infestations. One application was made on July 25, and the mite counts were made on August 2 and 15. All treatments except sulfur were superior to the check. Excellent control was obtained with demeton and good control with Rohm & Haas FW-293 and Aramite.

Experiment 2 was a combination of two tests, one on large and the other on small plots. On the large plots applications were made previous to mite infestation. The emulsion sprays were applied at about 18 gallons per acre at 200 pounds pressure, using 2 nozzles per row, the wettable powder at 35 gallons, and the dust at 38 pounds per acre. Applications were made on July 7 and counts were made on July 19, August 4 and 22. On the small plots the emulsions were applied at about 5 gallons, the wettable-powder sprays at 55 gallons, and the dust at 43 pounds per acre. Applications were made on August 12 after the mite infestation, and counts on August 16 and 24. The new systemic insecticides Am. Cyanamid 12008 and 3911, and the nonsystemic Stauffer R-1303 all gave excellent control and were superior to demeton and to General Chemical 928 applied either as spray or dust. Good control was obtained with demeton and poor control with compound 928.

Experiment 3 was a test of three materials applied on large plots late in the season, on August 18. The plants

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[2] M. W. Stone and F. B. Foley assisted in some of the field work reported in this paper.

Table 1.—Control of the two-spotted spider mite on lima beans in southern California, 1952–55.

Insecticide and Strength	Total Pounds of Toxicant per Acre	Mites per Leaflet: Average Number	Mites per Leaflet: Per Cent Reduction
1952 Experiment (6 replicates, 2 dust applications, 9 counts)			
Demeton 4%	1.6	3.7	83
Aramite 3%	2.1	3.9	82
Allied Chemicals 876[a] 20%	11.2	4.0	81
Ovex 10%	5.8	5.9	72
NPD 4%	2.4	8.9	58
Malathion 5%	3.2	9.8	54
Parathion 2%	1.3	16.0	25
DN-289,[b] 1.1%	.6	16.3	23
Allied Chemicals 876, 10%	5.7	17.1	19
Chlorobenzilate 2%	1.1	18.6	12
Sulfur 100%	64.0	21.2	0
Metacide 2%	1.3	21.8	0
L.S.D. at 5% level	—	11.0	52
1953 Experiment (6 replicates, 2 dust applications, 11 counts)			
Ovex 10%	5.8	3.4	73
5%	3.0	6.8	45
Chlorobenzilate 4%	2.2	8.2	34
Parathion 2%	1.2	8.4	33
Demeton 2%	1.2	9.6	24
Aramite 3%+sulfur 50%	1.9+32.5	11.3	10
Untreated	—	12.5	—
Malathion 5%	3.1	13.6	0
Aramite 3%	1.9	16.3	0
Sulfur 100%	65.0	33.0	0
L.S.D. at 5% level	—	15	—
1954 Experiment (8 replicates, 2 applications, 5 counts)			
Demeton 2 lb.,[c] 1–19 spray	1.0	7.2	94
Rohm & Haas FW-152, 33%, 1-29 spray	2.2	12.9	90
FW-293, 5% dust	3.3	25.7	80
Aramite 3% dust	1.9	27.9	78
Malathion 8% dust	4.9	46.7	65
Parathion 2% dust	1.3	63.6	51
Diazinon 4% dust	2.4	63.6	51
Holcomb 326, 25% spray 1-19	2.1	73.8	43
Ovex 10% dust	6.0	86.5	33
Chlorobenzilate 4% dust	2.4	87.0	32
Untreated	—	128.8	—
Sulfur 100% dust	75.0	133.0	0
L.S.D. at 5% level	—	28.4	22
1955 Experiment 1 (6 replicates, 1 application, 2 counts)			
Demeton 2 lb., 1-39 spray	0.3	1.8	99
Rohm & Haas FW-293, 5% dust	1.6	25.6	81
Aramite 3% dust	.9	30.1	77
Dow ET-15 5% dust	1.5	54.0	59
Hercules AC-528,[d] 2% dust	.9	49.7	55
Ovex 10% dust	3.6	65.7	51
Sulfur 100% dust	51.0	115.1	13
Untreated	—	133.0	—
L.S.D. at 5% level	—	41.4	31
1955 Experiment 2 (13 replicates, 1 application, 5 counts)			
Stauffer R-1303,[e] 25% WP, 3 lb., 100 gal. spray	.3	.6	96
Am. Cyanamid 12008, 4 lb., 1-99 spray	.5	.8	95
Stauffer R-1303, 4 lb., 1-99 spray	.5	1.0	94
Am. Cyanamid 3911, 4 lb., 1-99 spray	.5	1.1	93
Demeton 2 lb., 1-99 spray	.2	3.5	79
General Chemical 928, 2 lb.,[f] 1-13 spray	2.3	8.6	49
10% dust	4.0	14.0	18
Untreated	—	17.0	—
L.S.D. at 5% level	—	1.6	10
1955 Experiment 3 (7 replicates, 1 application, 3 counts)			
Demeton 2 lb., 1-55 spray	.2	16.4	81
Aramite 3% dust	.8	29.8	66
Aramite 2 lb., 1-13 spray	1.0	32.9	62
Untreated	—	87.3	—
L.S.D. at 5% level	—	15.8	18
1955 Experiment 4 (8 replicates, 1 application, 4 counts)			
Ovex 2 lb., 1-32 spray	3.02	1.82	97
Hercules AC-528, 2 lb., 1-99	1.00	2.45	95
Aramite 2 lb., 1-109	.80	4.30	92
Rohm & Haas FW-293, 1.6 lb., 1-52	1.51	6.72	87
Dow ET-15, 2 lb., 1-65	1.51	18.58	65
L.S.D. at 5% level	—	11.38	—
Untreated	—	53.53	—

[a] Bis(*p*-chlorophenyl) ethynyl carbinol.
[b] Triethanolamine salt of dinitrobutylphenol.
[c] Pounds indicate weight of insecticide in 1 gallon of emulsifiable concentrate.
[d] 2,3-*p*-Dioxanedithiol S,S-bis(O,O-diethyl phosphorodithioate).
[e] O,O-diethyl S-p-chlorophenylthiomethyl phosphorodithioate.
[f] 4-Chlorophenyl benzenesulfonate.

were heavily infested with mites. The sprays were applied at 550 to 600 pounds pressure using a 20-foot boom with 10 nozzles spread 2 feet apart, and the dust was applied with a power duster. Mite counts were made after 1, 4, and 12 days. Demeton gave quicker and better control than Aramite either as spray or dust, and all treatments were superior to the check. The treated plots yielded from 1.4 to 2.3 more 100-pound sacks of dry lima beans per acre than the check, but this difference was not statistically significant. This field was treated so late (about a month from cutting) that significant increases in yield were not anticipated, but it seems probable that they would have been obtained had it not been for the extremely hot weather 2 weeks following application which prematurely dried the vines.

Experiment 4 was a test of five materials applied as dilute sprays at 200 pounds pressure on June 27 and 28, and the mite counts were made July 13 to August 23. Very good to excellent mite control was obtained with ovex, Hercules AC-528, Aramite, and Rohm & Haas FW-293; and fair control with Dow ET-15. Mite populations were considered too low to affect crop yield, as most of the mites recorded were found onAugust 23, just a week before cutting. Yields (sacks of dry beans per acre) were as follows: ET-15 (16.2); FW-293 (15.8); Aramite (15.7); AC-528 (15.7); and ovex (13.0). Ovex was inferior to all others in yield, indicating an injurious effect on the beans. Marginal leaf burn was noted in the ovex spray plots.

TIME AND NUMBER OF APPLICATIONS (TABLE 2).—In 1952 dusting sulfur was applied at 2-week intervals on small plots in several schedules. The first application was made on May 26. Those made 2 and 4 weeks later were called the second and third applications although they may have been the only ones made. Eight counts were made at weekly intervals beginning on July 1. None of the dusted plots had significantly fewer mites than the check plots, and the increase in yield was not significant.

Table 2.—Effect of time and number of applications on control of the two-spotted spider mite on lima beans, 1952 and 1954.

Materials	Applications, at 2-week Intervals	Total Pounds of Dust per Acre	Mites per Leaflet		Number of Sacks per Acre
			Average Number	Per Cent Reduction	
1952 Experiment (6 replicates, 8 counts)					
Sulfur	First, third	51	47.2	0	30.8
	First	21	38.0	19	30.1
	First, second	51	43.5	7	30.1
	Third	31	66.2	0	29.8
Untreated	—	—	46.8	—	29.3
Sulfur	Second	30	59.9	0	28.4
	Second, third	51	68.1	0	27.9
L.S.D. at 5% level	—	—	19.7	—	[a]
1954 Experiment 1 (6 replicates, 4 counts)					
Aramite 3%	First, third	85	0.1	100	24.2
	First, second, third	125	0.2	100	23.9
	Second, third	88	0.3	100	23.8
	First	37	7.9	96	23.8
	First, second	77	1.1	99	23.2
Ovex 10%	Second, third	81	3.0	98	21.2
Aramite 3%	Second	40	10.7	94	21.0
Ovex 10%	Second	36	22.7	88	20.8
	Third	45	35.2	81	20.4
	First, third	78	7.0	96	20.4
	First, second, third	114	.8	100	20.1
	First, second	66	2.2	99	19.9
Aramite 3%	Third	48	2.9	98	19.8
Ovex 10%	First	33	37.3	80	19.7
L.S.D. at 5% level	—	—	14.7	8	3.5
Untreated	—	—	189.9	—	15.5
1954 Experiment 2 (5 replicates, 4 counts)					
Aramite 3%	First	28	0.1	100	29.6
	First, second	60	.1	100	28.6
	Second	32	.2	100	27.5
Untreated	—	—	41.4	—	24.6
Ovex 10%	First	28	3.2	92	24.2
	First, second	60	.8	98	23.2
	Second	32	6.9	83	20.0
L.S.D. at 5% level			—	—	4.4

[a] Not significant by F test.

However, there was a tendency for the first application alone to give better mite control than the second or third and for the first plus the second to be better than the second plus the third.

Experiment 1 in 1954 compared seven schedules of application of 3% Aramite and 10% ovex dusts on large plots. The same experiment was also conducted in 1953 and 1955, but since the mite populations were too low in those years to cause damage, the results are not included here. In 1954 the applications were made on May 28, June 11 and 25, and eight counts were made between June 3 and July 30. In mite control all treatments were superior to the check, and Aramite was superior to ovex. No significant difference was evident between single and multiple applications of Aramite, but multiple applications of ovex were superior to single ones. In yield all treatments were superior to the check, and Aramite was superior to ovex. There was no significant difference between the various schedules of ovex, but with Aramite the third application alone was inferior to all other schedules except the second alone. With single applications of Aramite, yield was greater with the first than with the second, and greater with the second than with the third, but the first alone was equal to any of the multiple applications. Most of the damage occurred in the 2 weeks following the first application, and control at this time was the most important factor in the increased yields.

Experiment 2 in 1954 compared three schedules of Aramite and ovex dusts on large plots. The applications were made on July 14 and 28, and there were four counts from July 22 to August 10. In mite control there was no significant difference between Aramite and ovex, but both were better than the check. In crop yield Aramite was superior to ovex, and the first application alone was better than the second alone. Two applications were not significantly better than one. As mite populations were relatively low in this field and yields with ovex were not superior to the check, the indications are that some unnoticed damage resulted from the use of ovex.

In these timing experiments the first application was made when there were about 10 mites per leaflet. In experiment 1 in 1954 the plants were in the four-leaf stage on May 28 and counts were made on the large primary leaves. Yields in the Aramite plots decreased with each 2-week delay in starting applications after May 28. In experiment 2 in 1954 the plants were nearly full-grown on July 14 and counts were made on secondary leaves. With both Aramite and ovex there was a reduction in yield when there was a 2-week delay in starting applications (from July 14 to 28). It seems evident that early treatment is desirable when there is an early infestation, if the most benefit is to be realized in increased yields. In experiment 2 an application on May 28 when no mites were present would probably have been too early. In a field with a late infestation some saving in labor cost can probably be obtained by making one treatment for the combined control of mites and lygus bugs about July 1, but prompt application for mite control is needed if the infestation is earlier.

MARGINAL INFESTATIONS (TABLE 3).—Mite populations usually begin building up at the edges of a field, and gradually move in. In order to determine the damage at various distances into one field, counts were made in 50-foot strips parallel to and 40 feet or more from the edge. Eight counts made from July 1 to August 21 showed the mite population to be highest near the edge of the field and to decrease gradually as the distance into the field increased. Damage was also greatest in the first strip, and decreased to the forth strip (190 feet), beyond which there was no reduction in yield of beans.

These results show that growers can save considerable money by treating the margins of their fields early in June. If all four margins are dusted to a distance of 190 feet, a little less than half the acreage in a 40-acre field will be treated, and about one-sixth the acreage in an 80-acre field.

DEMETON RESIDUES (TABLE 4).—In 1954 demeton was

Table 3.—Population of the two-spotted spider mite and yield of beans at various distances from the margin of the field, Smeltzer, Calif., 1952.

Feet from Road (West to East)	Mites per Leaflet	100-lb. Sacks per Acre
41–90	107	25.3
91–140	74	27.4
141–190	56	29.2
191–240	42	31.5
241–290	27	31.7
291–340	11	31.7
L.S.D. at 5% level	18	1.8

applied at about 3, 8, and 16 ounces per acre on July 6. Two pounds of bean plants for residue analysis[3] were pulled from these plots after 1, 7, 14, 21, and 28 days. The initial residues were high. Although they dissipated rapidly, they were still evident in measurable quantities after 28 days.

In another experiment in 1954 approximately 3, 7, and 13 ounces of demeton were applied on August 3, about 28 days before harvest, and bean pods were picked 1, 7, 14, 21, and 28 days after treatment, for residue analysis.[3] No residues were found except on whole pods picked after 7 days when analysis showed 0.2 p.p.m., which is below the official tolerance.

TESTS AGAINST *TETRANYCHUS DEVIATARSUS* MCG. (TABLE 5).—In 1953 one application was made to small plots on July 1, and seven mite counts were made between July 2 and August 11. There were no significant differences between treatments due to uneven infestations in the field, but ovex and malathion appeared to be ineffective against this mite.

In 1954 two applications were made to small plots on May 26 and June 18, and five mite counts were made between June 3 and July 26. Best results were obtained with Holcomb 326, Rohm & Haas FW-293, demeton, and Aramite.

In 1955 one application was made to small plots on July 12, and three counts were made from July 20 to August 16. Excellent control was obtained with demeton and FW-293, good control with sulfur and Aramite, fair control with Dow ET-15 and Hercules AC-528, and no control with ovex.

From these experiments over a 3-year period it seems apparent that ovex and malathion, which are fairly effective against the two-spotted spider mite, are ineffective against *T. deviatarus*, whereas with sulfur the situation is reversed. Demeton, FW-293, and Aramite are fairly effective against both species of mites.

SUMMARY.—In tests from 1952 through 1955 against *Tetranychus telarius* (L.), on lima beans in southern California, the following materials were the most promising: demeton; Am. Cyanamid 12008 and Am. Cyanamid 3911; Rohm & Haas FW-152 and Rohm & Haas FW-293; Stauffer R-1303; Hercules AC-528, Aramite, and ovex. Good to poor results were obtained with malathion, parathion, Holcomb compound 326, NPD, DN-289, Diazinon, Dow ET-15, Chlorobenzilate, Metacide, Allied Chemicals 876, General Chemical 928, and sulfur.

No control was obtained with one or two applications of dusting sulfur. With Aramite and ovex dusts, a single early application gave significant increases in yield, and was superior to a single application made 2 or 4 weeks later. Two or three applications were no better than the first alone. Later in the season the first application was superior to one applied 2 weeks later. Aramite was superior to ovex, and there was evidence that ovex caused crop reduction. In late infestations mite and lygus bug applications may be profitably combined, but in early infestations early application is essential for the best mite control.

Mite damage decreased progressively from the edge toward the center of the field up to 190 feet, and beyond this no measurable crop damage occurred; therefore, marginal treatments alone can result in substantial savings to the growers.

Demeton residues were found to be present in the whole plants 28 days after application, but in the beans and pods no residues were found at 14, 21, and 28 days, when dosages from 3 to 13 ounces per acre were used.

Table 4.—Demeton residues on lima bean plants at various intervals after treatment, 1954.

Ounces Applied per Acre	Parts per Million after (Days)				
	1	7	14	21	28
3.5	4.9	1.4	0.7	0.3	0.5
7.6	6.3	5.6	0.5	0.3	0.4
16.3	17.2	5.9	4.6	0.5	0.7

Table 5.—Control of *Tetranychus deviatarsus* on lima beans, Smeltzer, Calif., 1953–55.

Insecticide and Strength	Pounds of Dust or Gallons of Spray per Acre	Mites per Leaflet	
		Average Number	Per Cent Control
1953 Experiment (4 replicates, 1 application, 7 counts)			
Dusts:			
Parathion 2%	32	2.9	87
Aramite 3%	34	4.0	82
Chlorobezilate 4%	32	4.5	79
Aramite 3%+sulfur 50%	31	6.3	71
Aramite 3%	35	8.8	59
Malathion 5%	35	14.2	35
Ovex 5%	35	16.8	23
Untreated	—	21.8	—
Ovex 10%	36	26.5	—
L.S.D. at 5% level	—	[a]	—
1954 Experiment (8 replicates, 2 applications, 5 counts)			
Holcomb compound 326, 25%, 1-9 spray	19.6	2.7	84
Rohm & Haas FW-293, 5% dust	57	3.6	78
Demeton 2 lb.,[b] 1-19 spray	8.4	4.5	73
Aramite 3% dust	59	4.9	70
Rohm & Haas FW-152, 33%, 1-29 spray	20.6	8.1	50
Chlorobenzilate 4% dust	58	9.4	43
Sulfur 100% dust	61	11.2	31
Diazinon 4% dust	58	11.8	28
Parathion 2% dust	58	13.3	19
Untreated	—	16.4	—
Ovex 10% dust	57	29.2	0
Malathion 8% dust	59	50.3	0
L.S.D. at 5% level		10.6	
1955 Experiment (7 replicates, 1 application, 2 counts)			
Demeton 2 lb., 1-39 spray	5.7	1.2	98
Rohm & Haas FW-293, 5% dust	29	5.9	90
Sulfur 100% dust	31	11.0	81
Aramite 3% dust	29	15.7	73
Dow ET-15 5% dust	29	19.3	66
Hercules AC-528, 2% dust	35	26.1	54
L.S.D. at 5% level	—	10.8	—
Untreated	—	57.0	—
Ovex 10% dust	31	86.7	0
L.S.D. at 5% level	—	23.8	—

[a] Not significant by *F* test.
[b] Pounds indicate weight of insecticide in 1 gallon of emulsifiable concentrate.

[3] Analysis made by the Pittsburgh Coke and Chemical Co.

In tests against *T. deviatarsus* McG. excellent to good results were obtained with demeton, Holcomb 326, Rohm & Haas FW-293, Aramite, and parathion. Sulfur, which is ineffective against *telarius*, was fairly effective against *T. deviatarsus*, but the reverse was true for ovex and malathion. Demeton, FW-293, and Aramite were the only materials effective against both species.

REFERENCES CITED

Elmore, J. C. 1944. The control of the two-spotted mite on lima beans in California. U. S. Bur. Ent. & Plant Quar. E-613.

Wilcox, J., and A. F. Howland. 1954. Comparison of demeton dusts and sprays on beans and berries. Jour. Econ. Ent. 47(5): 945–6.

Spotted Alfalfa Aphid Occurrence on Seedling Alfalfa as Influenced by Systemic Insecticides and Varieties[1]

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The spotted alfalfa aphid (*Pterocallidium* sp.) has been a pest of New Mexico alfalfa since the fall of 1953,[3] when it first appeared in damaging numbers in the central Pecos Valley. By the following fall, this insect was epidemic on alfalfa throughout New Mexico, and it was evident that it was the major deterrent in the establishment of new seedings of alfalfa. Field observations made during the fall of 1954 and the spring of 1955 showed than an average of one aphid per seedling plant caused a severe reduction in stand.

The aphids attack the plants as soon as they emerge and may be found on the cotyledons and the hypcotyl. Currently, control on seedlings is obtained by conventional dusting or spraying shortly after emergence and again 10 to 14 days later. This practice has several serious disadvantages: The movement of heavy equipment over new seedlings damages the stand; the machinery available is poorly adapted for treating minute seedlings; under weather conditions prevailing at this time of year, coverage by either dust or spray is hindered by wind; low temperatures frequently further reduce the effectiveness. Since several applications are necessary to secure control, the cost of maintaining seedling stands is high.

These limitations to dusting and spraying necessitated the evaluation of other methods of applying insecticides. The use of systemics as seed or soil treatments as a means of overcoming certain of these undesirable features was investigated.

METHODS AND PROCEDURES.—The first experiment, planted March 17, was designed to evaluate both varietal resistance to the aphid and the effectiveness of different methods of application of a systemic insecticide.

This experiment, planted in rows on 18-inch centers, was set up in split-plot design, with the main-plot effect being assigned to the varieties and the sub-plot effect to the insecticidal treatments. The main plots or varieties were single rows 30 feet long and were divided into three 10-foot treatment sub-plots. There were 12 varieties of alfalfa (table 2) replicated six times and the plot area was bordered on all sides by untreated alfalfa.

Thimet[4] was used at the rate of 1 pound per acre, both as a seed treatment and in granulated form applied to the soil at planting time. The seeds to be treated were first sprayed with a solution of water and corn starch and then dusted in a dusting tower with the quantity of Thimet 44D required to deposit the desired amount on the seed. The addition of starch was necessary to improve adherence of the Thimet to the seed. This proved only partially satisfactory, since much of the Thimet rubbed off even with careful handling. However, since the seeds for each sub-plot were treated and packaged separately, the correct amount of chemical was applied to the seed furrow. After air drying, the seeds were packaged for planting.

The granulated Thimet was applied directly to the seed furrow immediately before seeding. The furrow was closed after seeding by covering with washed sand. A much more uniform stand was insured by covering the seed with sand rather than with native heavy adobe soil. Water was applied by flood irrigation the following day.

Stand counts were taken on April 25 from two 1-foot sections of each sub-plot. Aphid counts, likewise, were taken from two 1-foot sections of the sub-plots on May 30 and June 11. These were made by cutting the plants at ground level after carefully placing a cardboard apron underneath to avoid the loss of aphids which fell from the plants. The samples were placed in a small Berlese funnel and a small quantity of methylisobutylketone was placed on some cotton in the lid. The aphids left the plants and dropped into vials of alcohol. These samples were taken to the laboratory and counted.

The second experiment, planted on September 27, was similar in design to the first. Six varieties of alfalfa, selected on the basis of their tolerance to aphid attack, were used (table 5). Each variety row in this series was separated from the next by a buffer row of untreated New Mexico Common alfalfa.

The varieties were replicated six times, and each 40-foot variety plot was sub-divided into 10-foot sections to accommodate the four treatments. The treatments consisted of Thimet, Bayer 19639 and 23129[5] (all granulated

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[3] Not recognized as a new pest until the spring of 1954.

[4] Thimet was supplied by the American Cyanamid Company.

[5] The two Bayer compounds were supplied by Geary Research Laboratories. The formula for Bayer 23129 is *O,O*-dimethyl *S*-(4-oxo-3H-1,2,3,-benzotriazine-3-methyl) phosphorodithioate.

materials applied at 1 pound of actual toxicant per acre), and an untreated check. Granulated formulations of 2% Thimet[6] and 2% of the two Bayer compounds were employed in this experiment. The treatments were applied, seed planted, and the rows covered in the same manner as in the spring-planted plots.

Data on both aphid population and plant growth were obtained from this series. The aphid counts were made 28 days after seeding by carefully sliding 4- by 6-inch cards under the seedling plants from both sides of the row. Agitation of the plants caused the aphids to drop onto the cards, where they were counted in place. Each treated plot was sampled twice in this manner; thus, 12 inches of row were sampled in each plot.

The amount of plant growth per sub-plot was measured in grams 44 days after seeding. Samples were composed of all topgrowth plus 1½ inches of the underground portion of the stem and roots from two 7-inch portions of each sub-plot. The samples were placed in individual paper bags and taken to the laboratory, where they were washed and all dead material removed. The samples were than refrigerated at 35° F. until weighed.

A third experiment to determine the influence of Thimet and Bayer 19639 and 23129 upon emergence and growth of alfalfa seedlings was started in the greenhouse on August 1. The planting media consisted of a mixture of one part sand, one part adobe soil and one part peat moss. The granulated insecticides were applied at 4 pounds actual toxicant per acre in open furrows just prior to seeding. A randomized block design with 20 replicates was used. New Mexico Common alfalfa was used throughout, with each row receiving 50 seed.

RESULTS.—*Experiment 1.*—In the spring experiment, stand counts taken 39 days after seeding showed that Thimet, both as a seed treatment and as granules in the furrow, reduced the stand of seedling alfalfa of all varieties tested. The granules reduced stands more than the seed treatment (table 1).

The aphid population increased rather slowly in this experiment and appreciable numbers did not appear before early June. There was no significant difference in population on June 11, 56 days after seeding, as a result of any of the treatments. However, there was a difference in aphids present on the various alfalfa varieties. Lahontan, African, Ranger, Caliverde, New Mexico 17, Indian, and New Mexico 16 had significantly smaller populations than the standard, New Mexico Common, at the 5% level. The smaller number of aphids found on Caliverde was probably due to weakened plants which did not support a heavy infestation. Buffalo, New Mexico 11-1, Narragansett, and Talent had populations of aphids similar to that to New Mexico Common (table 2).

Table 1.—Effect of two Thimet treatments upon stands of seedling alfalfa,[a] 1956.

TREATMENT	AVERAGE NUMBER OF PLANTS PER SAMPLE[b]
Thimet, granulated	110.4
Thimet, seed treatment	117.4
Check	124.0
L.S.D. at 5% level	10.4

[a] Planted March 17. Data taken April 25.
[b] Composite of 12 varieties (2 feet of row per sample).

Table 2.—Spring populations of the spotted alfalfa aphid upon 12 varieties of alfalfa, 1956.

VARIETY[a]	AVERAGE NUMBER OF APHIDS PER SAMPLE PLOT
New Mexico Common	580.5
New Mexico 11-1	521.3
Narragansett	475.7
Talent	459.5
Buffalo	457.3
Indian	443.5*
New Mexico 17	441.5*
New Mexico 16	435.5*
Caliverde	397.2**[b]
Ranger	395.0**
African	334.0**
Lahonton	324.3**
L.S.D. at 5% level	124.8
L.S.D. at 1% level	166.4

* Significantly different from New Mexico Common at the 5% level.
** Significance as above at the 1% level.
[a] Planted March 17. Data taken June 4. (three samples)
[b] This figure is believed to be due to weakened plants which could not support a heavy aphid population.

Experiment 2.—In the fall experiment, population readings were taken 28 days after seeding during a heavy aphid attack. At this time, the plants in the buffer rows of New Mexico Common were almost completely killed out, as were those in the untreated check plots of this variety. The check plots of the New Mexico 11-1, African, and Ranger varieties were also severely damaged. A fair stand of Lahontan and New Mexico 16 survived in the checks, but the plants were definitely stunted.

The Thimet treated plots of all varieties were rather badly damaged. While the Bayer 23129 plots were also injured, there was substantially more plant growth than in the Thimet plots (table 3). Those treated with Bayer 19639 showed little or no injury, excellent stands of all varieties survived, and there was 60% more plant growth than on the next best treatment.

The aphid population ranged up to 93 per 12 inches of row. Since the check plots were either completely killed or badly injured, they could not maintain aphid populations. For this reason, data from them were not included in the analysis of the treatments. Analysis of the data from treated plots showed that the Bayer 19639 treatment had significantly fewer aphids than either the Thimet or the Bayer 23129 treatments (table 4).

The number of aphids found on the treated plots of the several varieties was tabulated. The checks as noted above were unable to support sizable aphid infestations. Of the six varieties in the experiment, only African supported a population as large as or larger than the standard. Ranger, N. Mex. 16, N. Mex. 11-1, and Lahontan supported populations significantly smaller than the standard, New Mexico Common (table 5).

The data on plant growth taken from the untreated checks, unlike the aphid counts from these plots, were analyzed along with those from the treated plots and show

[6] Analysis run in October–November, 1956, revealed that only 0.3% Thimet remained in the formulation.

Table 3.—Increase in growth of alfalfa seedlings due to insecticidal treatment. 1956.[a,b]

Treatment	Total Plant Growth in All Samples (grams)	Average Plant Growth per Sample (grams)
Bayer 19639	141.9	3.5**
Bayer 23129	86.2	2.4*
Thimet	40.8	1.1
Check	25.3	0.7
L.S.D. at the 5% level		1.4
L.S.D. at the 1% level		1.9

[a] Planted September 27. Data taken November 9.
[b] Sample included entire plants with 1½ inches of their roots from 14 inches of row.

Table 4.—Effect of three granulated insecticides on fall populations of the spotted alfalfa aphid. 1956.[a,b]

Treatment[c]	Average Number Aphids per Sample[d]
Thimet	187.8**
Bayer 23129	178.8**
Bayer 19639	25.7
L.S.D. at the 1% level	19.3

** Significantly different from Bayer 19639 at the 1% level.
[a] Because few plants in the untreated plots survived the aphid attack, data taken from the check plots are not included.
[b] Planted September 27. Data taken October 25.
[c] All materials applied at 1 pound actual toxicant per acre.
[d] Composite of six varieties (1 foot of row per sample).

clearly that the plots treated with the Bayer compounds produced a significant increase in growth compared with the untreated check. The Bayer 19639 treatment gave outstanding results with nearly six times as much growth as the check, which proved to be a significant difference at the 1% level. The Bayer 23129 treatment was intermediate in its effect, with about three times as much growth as the check, and this difference was significant only at the 5% level. The growth of the Thimet-treated plots was about 1½ times that of the untreated check, but this difference was not significant (table 3).

In a separate analysis of the plant growth from only the check plots, a striking variation was shown in the

Table 5.—Fall populations of the spotted alfalfa aphid on six varieties of alfalfa.[a] 1956.

Variety	Average Number Aphids per Variety Plot (3 Samples)[b]
African	138.5
New Mexico Common	124.5
New Mexico 11-1	101.0**
Lahontan	81.3**
New Mexico 16	75.8**
Ranger	67.5**
L.S.D. at the 1% level	18.87

** Significantly different from New Mexico Common at the 1% level.
[a] Planted September 27. Data taken October 25.
[b] Check plots not sampled due to extreme injury by the aphids.

Table 6.—Plant growth of six varieties of alfalfa having no protection from a heavy attack by the spotted alfalfa aphid. 1956.

Variety[a]	Average Weight of Plant Growth per Sample[b] (grams)
New Mexico 16	1.62**
Lahontan	1.46**
Ranger	0.48
African	0.37
New Mexico 11-1	0.19
New Mexico Common	0.12
L.S.D. at the 1% level	1.26

** Significantly different from New Mexico Common at the 1% level.
[a] Planted September 27. Data taken November 9.
[b] Sample includes entire plants with 1½ inches of their roots from 14 inches of row.

amount of growth produced by the different varieties under heavy aphid attack. New Mexico 16, a variety developed by the Agronomy Department of New Mexico Agricultural Experiment Station, and Lahontan produced growth significantly higher than the standard, New Mexico Common. Growth of the other three varieties, Ranger, African, and New Mexico 11-1, was not significantly greater than the standard variety (table 6). The data indicate that the protection derived was from inherent tolerance and that the protection provided by the systemic insecticides was additive. No interaction was demonstrated.

Experiment 3.—Analysis of the emergence data showed that, when compared with the check, a significant reduction in stand resulted when the systemic insecticides were applied at four times the usual dosage applied. Greater reduction in stand resulted from the Thimet treatment

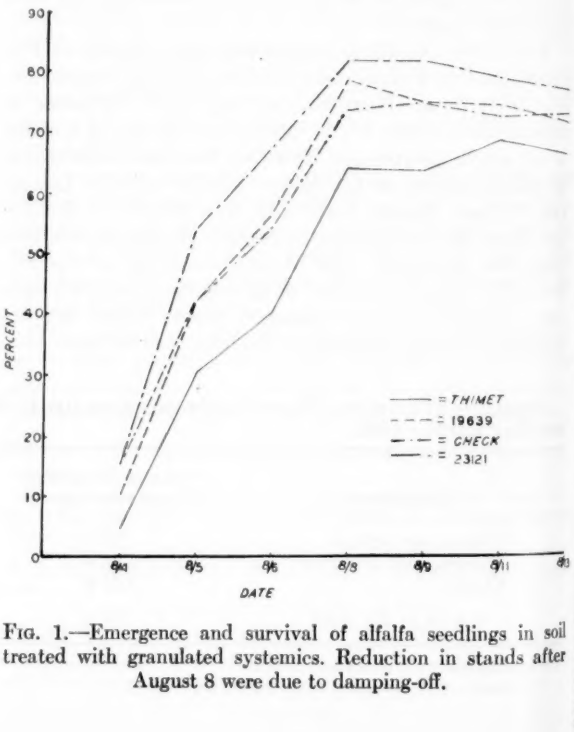

FIG. 1.—Emergence and survival of alfalfa seedlings in soil treated with granulated systemics. Reduction in stands after August 8 were due to damping-off.

than from the Bayer compounds. The injury caused by the latter was significant, but did not appear to be enough to reduce field stands materially (fig. 1).

SUMMARY AND CONCLUSIONS.—Thimet in granulated form and as a seed treatment was studied to determine its effect upon stands of alfalfa and populations of the spotted alfalfa aphid, *Pterocallidium* sp. Bayer compounds 19639 and 23129 as granulated materials were similarly tested.

Different alfalfa varieties were studied to determine whether the tolerance exhibited by mature plants was expressed in the seedling stage.

From the data obtained, it is evident that Thimet as a seed treatment, or as granulated material, and Bayer 23129 in granulated form are relatively ineffective in preventing spotted alfalfa aphid injury to seedling alfalfa. Bayer 19639 shows definite promise of protecting fall stands of seedling alfalfa for at least 6 weeks after planting.

Several varieties of alfalfa had less aphids and produced more vegetative growth than the standard, New Mexico Common. A new synthetic variety, New Mexico 16, and Lahontan performed best in this regard and were significantly better than New Mexico Common.

The Habits and Control of the Clover Mite in Dwellings[1]

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The clover mite, *Bryobia praetiosa* Koch, is not only an orchard pest but also a household pest as well. Huber (1910) discussed an invasion of a dwelling in Dresden, Germany, by clover mites in 1863. In this country, Webster (1912) reported clover mites entering dwellings literally in swarms at Lafayette, Indiana, as long ago as 1888. Although there are scattered reports in the literature of clover mites during the intervening years, it was not until the housing "boom" following World War II that the invasion of dwellings by clover mites became generally regarded as a serious problem. The recent upswing of the clover mite problem was associated by the junior author with new housing developments and luxuriant well-fertilized lawns growing close to the foundations of dwellings. The relationship between lawn grass and clover mite infestations in dwellings was also made by Britton (1931), Strickland (1952), and Mathys (1954).

Clover mites which enter dwellings through cracks in the floor, walls or masonry do not feed on the building and its contents, but may leave stains where they are crushed. They do not injure people or pets, but are considered a nuisance by many home owners, particularly when tremendous numbers infest the walls, windows, and floors.

Although acaricides have been used for the control of clover mites and various remedies have been proposed, there seems to be no published data on the control of this pest in and about houses. Following the discovery by the junior author that clover mite infestations about the base of elm trees behaved similarly to infestations in dwellings, a way was opened for the experimental work reported in this paper.

BIOLOGY OF THE CLOVER MITE.—The behavior described here is applicable only to clover mites infesting dwellings and lawn trees, because in other habitats the mites behave somewhat differently. The clover mite appears to show thelytokous parthenogenesis. At any rate, no male clover mites were found during the course of this investigation. The dark red mature female mite lays bright red eggs in cracks and faults in concrete foundations, in mortar crevices, on building paper between the walls of buildings, and on the underside of the basal bark of trees. In these places an unbelievable accumulation of eggs, egg shells, and molt skins can often be found. Optimum development of the egg and hatching occur during periods when the humidity is high and the temperature of the oviposition site falls within the range of 45° to 65° F. Below 45° F. the rate of development decreases until at near freezing temperature no hatching occurs. At temperatures above 65° F. the rate of development increases, but the per cent of eggs hatching decreases until at about 85° F. the eggs become dormant and do not hatch. For this reason few mites are seen around dwellings during the summer months. Dormancy of the eggs is broken only with the return of lower temperatures and higher humidities.

The newly hatched six-legged, crimson-colored clover mite larva migrates from the dwelling or tree to the lawn to feed on grasses, clovers, and lawn weeds. After feeding, the engorged larva returns to the dwelling or tree to molt. Following this molt, the protonymph migrates to the lawn to feed and then returns again to the dwelling or tree bark to molt. The deutonymph goes through the same circuit. The adult female behaves in a similar manner, migrating to the lawn to feed and back to the dwelling to oviposit. This feeding and egg-laying cycle may be repeated a number of times. When conditions become unsuitable, following the mowing of the lawn, or the approach of cold weather, the mites tend to accumulate beneath the basal bark of trees, or in cracks and crevices of buildings. Here they remain until conditions become favorable again or until they die. It is this search for protective sites for oviposition, molting, hibernation, and feeding that leads clover mites to accidentally enter dwellings and thereby become a nuisance.

The clover mite oversummers in the egg stage. With a

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[2] The authors are indebted to Mr. Robert S. Chamberlin of the Physical Plant Department of the University of Illinois for allowing the use of elm trees in certain areas of the campus; to Mr. Herbert Plant of the American Community Builders, Inc., Park Forest, Illinois, for the use of residential units and for assistance at various times; to Messrs. H. E. Jennings and Wesley Tryka of the Smithereen Company, Chicago, for cooperation during the initial stages of the work and for help in carrying out some of the first experiments.

Table 1.—Clover mite eggs and live mites on bark samples from the base of elm trees receiving various control treatments.

Treatment April 7 & 26	Eggs and Live Mites[a] per 100 Grams of Bark														
	1955													1956	
	4/5	4/14	4/21	4/30	5/6	5/17	5/31	6/21	7/25	8/24	9/27	11/12	12/20	2/14	3/13
Control	1290 (359)	447 (325)	847 (285)	954 (239)	2630 (254)	2820 (438)	1885 (637)	1095 (114)	200 (2)	331 (0)	380 (212)	5260 (896)	7680 (322)	12000 (1530)	14170 (1015)
Aramite spray[b]	1582 (470)	353 (83)	512 (176)	402 (16)	165 (13)	27 (18)	97 (1)	21 (2)	10 (3)	11 (0)	26 (17)	3390 (323)	3630 (87)	2300 (130)	7440 (257)
Aramite dust[c]	1042 (270)	683 (235)	546 (170)	483 (1)	331 (28)	13 (15)	80 (10)	113 (22)	43 (10)	8 (1)	118 (51)	3070 (344)	5764 (224)	4340 (565)	5550 (782)
Malathion spray[d]	780 (245)	205 (113)	211 (144)	338 (6)	140 (114)	39 (30)	798 (147)	636 (570)	31 (1)	118 (2)	43 (16)	2960 (453)	6980 (155)	3290 (572)	7250 (605)
Malathion dust[e]	1380 (243)	154 (69)	507 (288)	530 (5)	157 (106)	8 (11)	181 (17)	364 (63)	70 (2)	22 (0)	305 (246)	2390 (205)	5670 (293)	5360 (332)	7180 (291)
Grass removed 18"	412 (314)	278 (228)	288 (121)	583 (103)	818 (98)	255 (168)	250 (83)	75 (59)	11 (1)	27 (0)	18 (5)	908 (192)	1410 (23)	3200 (131)	1340 (44)

[a] Numbers given in parenthesis.
[b] Aramite 15-W, 4 lbs./100 gallons water.
[c] Aramite 15-W, about 1 lb. per tree, April 7; about 2 lbs. April 26.
[d] Malathion 25-W, 4 lbs./100 gallons water.
[e] Malathion 25-W, about 1 lb. per tree, April 7; about 2 lbs. April 26.

drop in temperature, often in late September, the eggs start hatching. The mites feed and the eggs continue to hatch until stopped by cold weather. All stages of the clover mite over-winter. Hatching and feeding occur on sunny days during the winter on the south and east sides of dwellings and trees. With the return of spring, mite activity increases and continues until early June, when the mites die and the eggs become dormant.

Migration of the Clover Mite.—To learn something about the distance the clover mite migrates from a protective site to a feeding site, aluminum bands coated with Tree Tanglefoot were placed in the grass at various distances from the base of a tree with a high population of clover mites under its basal bark. The daily record of the mite catch on 7 warm, bright, days in April 1955, indicated that 53% of the mites fed within 6 inches of the tree, 70% within 12 inches, and 95% within 24 inches of the tree. This observation suggested the possibility of controlling or, at least, reducing the mite population by removing a band of grass at the foundation of buildings. Strickland (1952) reported satisfactory control by this method in Canada.

Control Experiments.—From the behavior studies discussed above, it was evident that elm trees growing in the well-kept lawns on the campus of the University of Illinois might provide a number of convenient experimental units.

In the first of several experiments, the objective was to compare the effectiveness of acaricidal applications with a grass-free band. Aramite and malathion were applied as sprays with a power sprayer and as dusts with a rotary hand duster. The acaricides were applied about 10 feet up the trunk of each tree and for a distance of 2 feet away from its base. Each treatment was applied to three trees, selected in random fashion. Since showers, aggregating 3.14 inches of rain, occurred shortly after the first acaricidal application on April 7, the treatments were repeated April 26. Before treatment and at intervals afterwards about 25 grams of bark were taken from each tree for mite and egg counts.

Following the spray and dust treatments on April 26, there was a decided drop in the number of clover mites (table 1). The Aramite spray appeared to be the best of the treatments in keeping the mite population down through the remainder of the spring. The Aramite and malathion dusts were about equally effective, but the

Table 2.—Clover mite eggs and live mites on bark samples from the base of elm trees surrounded by grass-free barriers of various widths.

Treatment Aug. 15, 1955	Eggs and Live Mites[a] per 100 Grams of Bark														
	1955					1956									
	Aug.	Sept.	Oct.	Nov.	Dec.	Feb.	Mar.	Apr.	May	June	Sept.	Oct.	Nov.	Dec.	Total
Tall grass	494 (2)	815 (3)	2340 (144)	3740 (318)	4680 (363)	4880 (308)	5800 (463)	5460 (354)	8220 (370)	5530 (49)	5240 (6)	13200 (324)	18475 (1054)	13180 (1880)	92054 (5638)
Clipped grass	612 (2)	920 (21)	2310 (142)	4870 (673)	4580 (237)	6280 (162)	6420 (765)	5160 (605)	3500 (297)	3410 (13)	3832 (12)	6560 (165)	16131 (960)	17400 (1945)	81985 (5999)
Grass removed 6"	518 (1)	660 (2)	875 (88)	1550 (538)	2810 (78)	2620 (80)	2200 (95)	2260 (163)	2180 (87)	2640 (8)	2100 (2)	4500 (104)	9705 (613)	3050 (746)	37668 (2605)
Grass removed 12"	536 (3)	957 (9)	642 (87)	1320 (265)	2280 (45)	1820 (47)	1590 (88)	900 (141)	2800 (80)	828 (34)	1025 (0)	2360 (91)	1985 (277)	3100 (460)	22143 (1627)
Grass removed 24"	724 (0)	801 (2)	718 (40)	774 (103)	1053 (15)	1540 (22)	568 (98)	189 (67)	800 (61)	678 (2)	822 (0)	1510 (23)	1608 (130)	1265 (165)	13050 (728)

[a] Numbers given in parenthesis.

malathion spray did not hold up so well, although the differences are of doubtful significance. Surprisingly the effect of the acaricides carried through until the following March, when observations were discontinued. But the point of main interest was the gradual decline of the mite population on the block of trees with 18 inches of grass removed around the base.[3] While not so dramatic as the acaricidal treatments, the results in the long run were better, and decidedly so, by March of 1956.

The long-lasting effect of the acaricides is manifest in the egg counts, as well (table 1). Even more striking is the low level of the egg population at the base of trees with 18 inches of grass removed. The maintenance of a relatively low number of eggs that might well be deposited on or in the walls of a building is an extremely important factor in the economic control of the clover mite.

With the promising results from the above experiment it became important to verify the effectiveness of the grass-free band. Accordingly, a block of 20 trees of about uniform size, in a well-maintained lawn, was selected for further tests. An 18-inch band of grass was removed at the base of 10 trees, while the other 10 were left as controls. The arrangement of the trees was in pairs. Mite counts were started in April 1955 and carried through

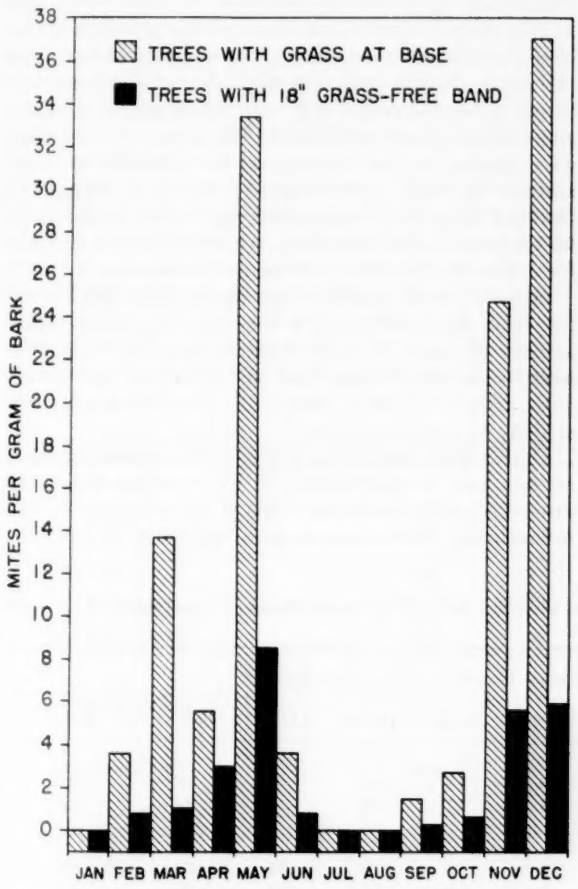

FIG. 1.—Graph showing the contrast in the number of clover mites counted on bark samples from elm trees with grass at their base and from trees surrounded by an 18-inch grass-free band. There were 10 trees in each group.

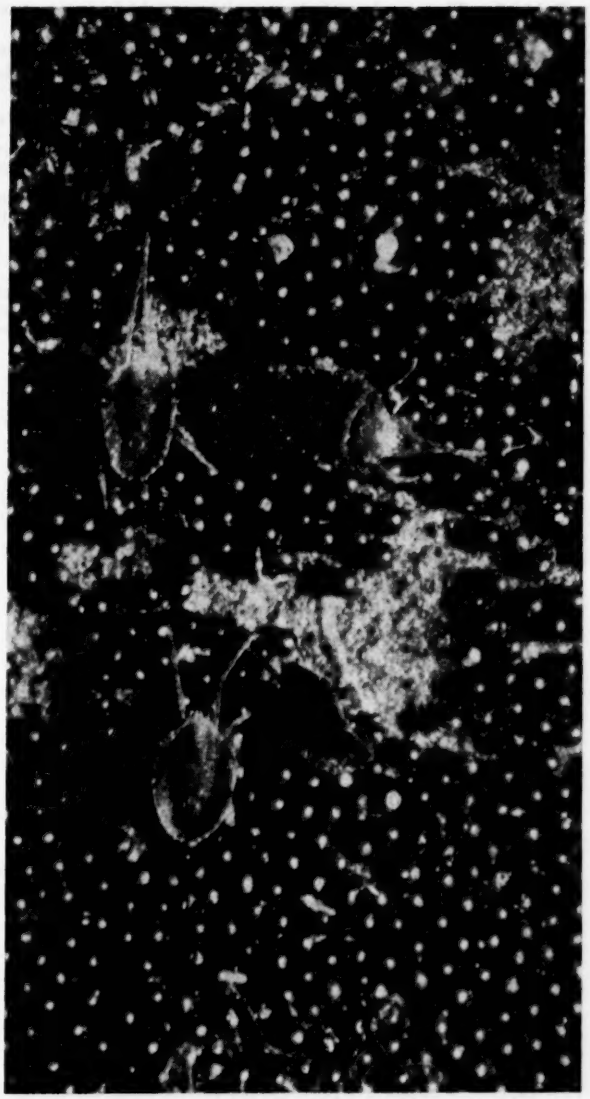

FIG. 2.—Clover mites and their eggs on the underside of a piece of bark. Greatly enlarged.

December 1956. Data for the 2 years were combined in the graph (fig. 1). These data show a striking contrast between the clover mite populations on the bark from the trees with grass and from the trees without grass. The mean population ratio on trees with grass to those without grass was 4.8 to 1.

EFFECTIVENESS OF GRASS-FREE BANDS OF VARIOUS WIDTHS.—This experiment was designed to determine the clover mite populations on the basal bark of elms surrounded by tall grass (enclosed in a fence), clipped grass, and grass-free bands 6, 12, and 24 inches wide around the trees. A long row of elms, about 18 inches in diameter, growing in a grassy lane was chosen for the experiment. The trees were examined for mite populations before the experiment was set up. Five trees were used for each treatment randomized in five blocks. Circles

[3] In all the experiments the grass-free bands were maintained by occasional hoeing.

Table 3.—The number of clover mites caught in traps at windows of apartments with grass and with grass removed for 18 inches at the foundations.

Treatment	Number of Mites in Traps on Dates Below																
	1955						1956										
	8/22	9/20	10/8	10/25	11/22	12/13	3/20	4/24	5/15	6/5	7/17	8/22	9/18	10/16	11/13	12/18	Total
Grass removed	25	18	159	34	56	3	0	17	43	57	45	9	12	20	0	6	504
Grass	17	4	148	141	158	40	10	382	186	85	12	0	0	0	34	105	1322
Grass removed		—[a]					17	139	180	83	11	1	0	6	19	19	475
Grass							14	440	363	195	30	0	0	1	62	41	1146
Grass removed	16	2	88	31	8	0	2	2	5	31	21	0	1	0	0	1	208
Grass	26	6	33	82	102	49	33	—	23	603	13	0	1	3	7	20	1001
Grass removed[b]	41	20	247	65	64	3	19	158	228	171	77	10	13	26	19	26	1187
Grass[b]	43	10	181	223	260	89	57	822	572	883	55	0	1	4	103	166	3469

[a] One of these units was relocated because of interference by tenants.
[b] Totals.

around the trees were made with lime water so that the grass could be removed for the proper distance. Beginning in August 1955, bark samples amounting to about 10 grams per tree were taken at monthly intervals (excepting January, July and August 1956) for counts of mites and eggs.

As indicated by mite counts and egg counts (table 2), there was little difference between the mite population on the bark of trees surrounded by tall grass and trees surrounded by clipped grass. However, the removal of only 6 inches of grass cut the mite population roughly by one-half. The reduction in the population was essentially in proportion to the width of the grass-free band. It will be observed that during October, November, and December 1956 (a more favorable mite season than 1955), there were about 10 times as many eggs on the bark of trees surrounded by tall grass as on trees with the 24-inch grass-free barrier. Thus, the effectiveness of the grass-free band in reducing the winter carry-over and the spring mite potential was demonstrated again.

EXPERIMENTS WITH DWELLINGS.—Through the cooperation of American Community Builders, Inc., Park Forest, Illinois, arrangements were made to use some of their apartment rental units for tests with grass-free bands. The units chosen had eight apartments each with two picture windows, where clover mites were most frequently observed. The units were about 24×140 feet in size and of similar, but not identical, structure. Three pairs of units were selected in three locations of the housing development where observations and tenant complaints indicated trouble with clover mites. Each unit of a pair was parallel to the other and only a short distance from it. The grass was removed for 18 inches at the foundation of one unit in each pair and the other unit was used as a control.

To provide quantitative data, traps were placed just under the frame of each picture window on the outside of the building. The traps were made by placing a piece of 1-inch masking tape about 8 inches long on the wall and by running a $\frac{1}{8}$-inch strip of Tree Tanglefoot on the tape with a syringe. While many mites were caught in the Tanglefoot, more were caught under the edges of the tape as they moved in the cracks and crevices provided by the irregularities in the outside wall. It was soon evident that the most effective trap was made by pressing the tape firmly down the center line with the finger, leaving the edges rather loosely attached to the structure. New traps were applied to the buildings at 3- to 4-week intervals and the old traps were removed from the buildings and fastened to pieces of corrugated paper with thumb tacks for transport to the laboratory, where they were examined for mites with the aid of a binocular microscope.

Sixteen records made between August 1955 and December 1956, show, with only a very few exceptions, higher catches of mites in traps from apartments with grass next to the foundations than in traps from apartments with grass-free bands (table 3). The differences were striking in some instances.

During 1956, traps were placed at the foundation level on one pair of apartments, 10 to 12 inches above the ground, in addition to the traps at the windows. It is of interest that there was no great contrast between the

Table 4.—The number of clover mites caught in traps at the windows and at the foundations of apartments with grass and with grass removed for 18 inches.

Treatment and Location of Traps	Number of Mites in Traps on Dates Below, 1956									
	4/24	5/15	6/5	7/17	8/22	9/18	10/16	11/13	12/18	Total
Grass removed										
Window	17	43	57	45	9	12	20	0	6	209
Foundation	121	19	44	16	3	17	54	0	24	298
Grass										
Window	382	186	85	12	0	0	0	34	105	804
Foundation	3137	575	745	61	0	2	18	230	428	5196
Total, grass removed										507
Total, grass										6000

Table 5.—The summary of a clover mite survey made at Park Forest, Park Ridge, and Niles, Illinois, May 18 and 19, 1956.

Direction of Window	Average No. Mites per Window Over	
	Grass or Clover	Other[a]
South	40.6	2.8
West	31.2	0.2
North	35.0	0.1
East	71.4	1.2

[a] Flowers, weeds, or bare soil.

number of mites trapped at the window and the number trapped at the foundation levels on the unit surrounded by the grass-free band, whereas in traps from the unit with grass to the foundation, about six times as many mites were caught at the foundation level as at the window level (table 4). A high catch of mites at the foundation level, where the siding meets the concrete, indicates the magnitude of the mite population that may enter the building at this point not only to molt, oviposit, and find protection, but also to increase and ultimately reach the inside of the dwelling.

A Clover Mite Survey.—The experimental results presented above were further substantiated by a survey of 22 dwellings in three relatively new housing developments in northern Illinois. A count of the mites at 213 windows in May 1956 showed the almost complete absence of mites at windows where there was no grass, compared with counts of 31 to 71 mites at windows above grass or clover (table 5).

Further Control Experiments.—With additional knowledge at hand, it seemed advisable to make further tests with acaricides and the grass-free band. Thirty elms growing in a row along a grassy roadside were selected for five treatments and a control each of which was replicated five times. Aramite, Chlorobenzilate, and Ovotran were applied with a power sprayer to the tree trunks upwards for about 8 feet and to the grass extending outwards from the trunk for a radius of 8 to 10 feet. About 5 gallons of spray were used per tree. The spray dates, April 5, and September 20, were based on expected mite activity. Due to the long, dry fall of 1956, the September spray might have been applied 3 or 4 weeks later to better advantage.

That all treatments were effective is clear from the data given in table 6. The 18-inch grass-free band was almost as effective as two treatments with Aramite 15-W, or Chlorobenzilate 25-W, or Ovotran 50-W, used at the rate of 8 pounds per 100 gallons of water. The grass-free band supplemented with two Aramite sprays was the most effective treatment. It is entirely possible that such a combination treatment might be needed at first where clover mite populations are extremely high and when immediate relief is needed.

Clover Mite Control Inside Dwellings.—No satisfactory measure for the control of mites by the local application of materials inside houses was developed. At the beginning of this project, when little was known about the habits of the clover mite, such materials as Aramite 15-W, Chlorobenzilate 25-W and pyrethrum powder were dusted about infested window sills, baseboards, etc., for the immediate relief of tenants occupying mite-infested apartments. Results were qualitative rather than quantitative. Such treatments provided what appeared to be control for a few days up to several weeks. In the light of later experience it is evident that such measures inside a house could provide no more than temporary control, without outside measures. The frequent use of a vacuum cleaner should not be overlooked.

Discussion.—From the experimental work reported herein, it seems plain that removal of the grass near the foundations of buildings is the first step in combating an invasion of clover mites. The removal of only a 6-inch band of grass will help, but wider bands should be used if possible. A band 18 inches wide was considered adequate for practical use, but a 24-inch or wider band might be desirable in some situations.

Removal of the grass and clover at the foundation does

Table 6.—Clover mites and eggs[a] on bark samples from elm trees receiving various control treatments.

Treatment	Mites and Eggs[a] per 100 Grams of Bark, on							
	3/27	4/19	5/17	6/14	9/12	10/18	11/15	12/12
Control	221 (4770)	375 (3400)	54 (713)	0 (876)	0 (687)	40 (2248)	1667 (4296)	1361 (8254)
Aramite 15-W[b]	269 (4930)	79 (1440)	11 (134)	0 (116)	0 (16)	0 (12)	12 (68)	2 (129)
Chlorobenzilate 25-W[b]	275 (5340)	112 (1670)	18 (133)	0 (83)	2 (45)	0 (2)	23 (159)	23 (165)
Ovotran 50-W[b]	288 (5280)	26 (1930)	5 (236)	0 (370)	0 (149)	1 (83)	57 (624)	29 (919)
Grass removed 18″	252 (5160)	158 (1115)	16 (72)	0 (92)	0 (42)	3 (127)	110 (122)	86 (243)
Grass removed & Aramite[b]	236 (5140)	57 (1090)	3 (38)	0 (39)	0 (16)	0 (8)	8 (93)	0 (35)

[a] Numbers given in parenthesis.
[b] Eight lbs. per 100 gallons water, applied April 5, and September 20.

not mean that this area need be left bare and ugly. A variety of flowers, such as zinnia, marigold, salvia, rose, chrysanthemum, and petunia, which are not attractive to the clover mite, can be substituted for the grass. Likewise such shrubs as barberry, juniper, spruce, arborvitae, and *Taxus* spp. can be used without providing food for the clover mite. With some forethought, the builders of new houses, especially large developments, could save their tenants or buyers a lot of trouble with clover mites by leaving a bare strip of soil at the foundation in the beginning.

It may be necessary for quick results in some situations to supplement the grass-free band with acaricidal treatments. Such treatments without the grass-free band will provide temporary, or at best only seasonal control. If rain follows within a few days after the acaricide is applied it may be necessary to repeat the treatment, since the deposit of acaricide is not only depleted by the rain, but the conditions provided by the rainfall are favorable to the mites. If acarcides are necessary, they should be applied about April 1, and October 1, in the latitude of central Illinois. Results from acaricidal applications in late May are likely to be deceptive because of the natural decline of the mite population in early summer. Several

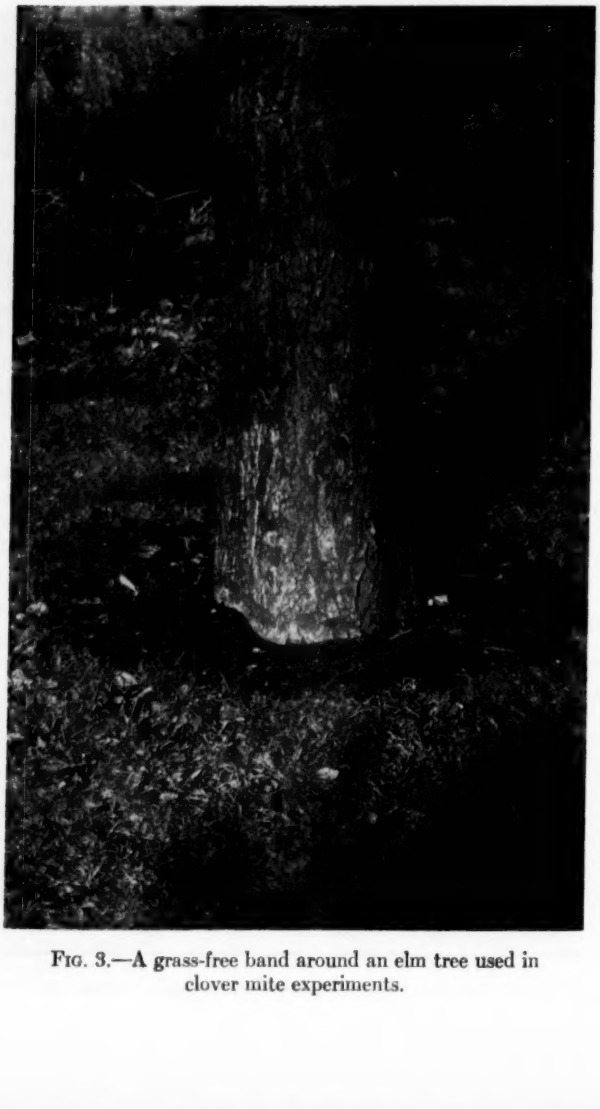

FIG. 3.—A grass-free band around an elm tree used in clover mite experiments.

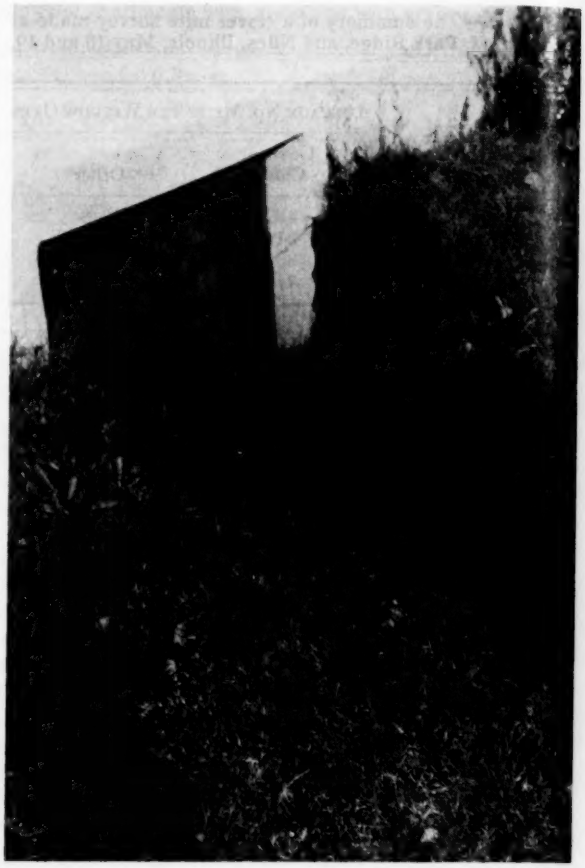

FIG. 4.—Favorable clover mite habitat at the foundation of a building.

excellent acaricides were available for these experiments but an arbitrary selection was made for practical reasons Such highly toxic materials as parathion were ruled out as undesirable for use in a community thickly populated with small children. Although malathion has been used by the pest control industry for some time it was ruled out because of its odor[4] and because its effectiveness was clearly no better than materials with less odor. While home owners may tolerate an objectionable odor in order to obtain relief from clover mites, tenants who do not pay for the treatment and who expect their apartments to be pest free are not likely to tolerate objectionable odors.

The residual action of acaricides should be increased by the use of high dosages and adequate gallonage, if sprays are applied. The recommended dosage for orchard use might well be doubled or quadrupled without injury to grass. Wettable powders were chosen for experimental use in place of emulsifiable concentrates since such concentrates are usually more phytotoxic than wettable powders. One way of obtaining a high dosage, used in early experiments with satisfactory results, was to apply wettable spray powders dry by means of a 2-wheel lawn fertilizer distributor. This method is quick and easy, but leaves a conspicuous deposit on the grass that may be

[4] More recently malathion has been greatly improved in this respect.

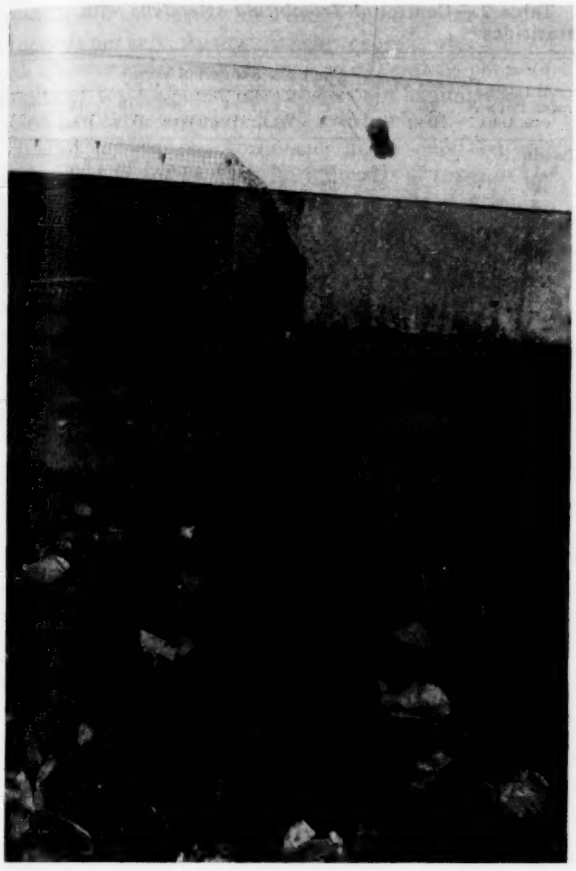

FIG. 5.—Unfavorable clover mite habitat created by removing the grass at the foundation of a building.

objectionable. Another method used was to mix the wettable powder with water and apply it with an ordinary sprinkling can.

SUMMARY.—The clover mite, *Bryobia praetiosa* Koch, a household nuisance associated with well-kept lawns, exhibits the same sort of behavior at the base of lawn trees that it does near the foundations of buildings. During the course of its development, the mite migrates back and forth between the lawn grass to feed and the building or tree to oviposit, molt, and seek protection. High summer temperatures are unfavorable to clover mite development and the summer is passed as a dormant egg. Climatic conditions in the spring and fall are particularly favorable and all stages may be observed during the winter.

Taking advantage of the behavior of the clover mite, trees were used as experimental units for a comparison of the effectiveness of grass-free bands and acaricides. Data obtained from bark samples and from mite traps on dwellings showed that grass-free bands around trees and buildings greatly reduced the mite population. The degree of control increased as the width of the band was increased from 6 to 24 inches. An 18-inch band was essentially as effective as applications of Aramite, Chlorobenzilate, malathion, and Ovotran. The grass-free band was more lasting in effectiveness than the acaricides, but the grass-free band supplemented with an acaricide spray gave the quickest and most nearly complete control.

REFERENCES CITED

Britton, W. E. 1931. Abundance of Clover Mite *in* Connecticut State Entomologist Thirtieth Report, 1930. Bull. Connecticut Agric. Expt. Sta. **327**: 574–5.

Huber, J. C. 1910. Bibliographie der Klinische-Entomologie (Hexapoden, Acarinen) Heft **2**: 1–24. Hermann Pohls. Jena, Germany.

Mathys, G. 1954. Contribution éthologique à la résolution du complexe *Bryobia praetiosa* Koch (Acar., Tetranych.). Mitteil., schweiz. ent. Ges. **27**(2): 137–46.

Strickland, E. H. 1952. Insects of the year in Northern Alberta, 1951 *In* The Canadian Insect Pest Rev. **30**(1): 22–5.

Webster, F. M. 1912. The clover mite *Bryobia pratensis* Garman. Jour. Econ. Ent. **5**(3): 290–5.

Small-Plot Tests for the Control of Chiggers[1,2]

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Chiggers, or red bugs, are not only pests of man, but in the Asiatic Pacific area they are vectors of scrub typhus and suspected vectors of hemorrhagic fever. These diseases caused many casualties during World War II and the Korean conflict.

Clothing repellents, such as benzyl benzoate, provide excellent individual protection against chiggers, but it is often preferable to eliminate the pests from areas that will be continuously or repeatedly occupied by troops, vacationers, or woodsmen.

Linduska *et al.* (1948) obtained good control of chiggers in Florida with BHC and several other insecticides at dosages of 25 to 100 pounds of the toxicant per acre. Smith & Gouck (1947) found chlordane, toxaphene, and BHC to be effective at 2 to 8 pounds per acre. Traub *et al.* (1954) obtained excellent control of chiggers in Borneo with dieldrin and aldrin at 2¼ pounds per acre.

From 1947 to 1954 several promising acaricides that were available in sufficient quantities were tested in the field near Orlando, Fla., against *Eutrombicula alfreddugesi* (Oud.) or *Trombicula splendens* (Ewing). The tests against *alfreddugesi* were conducted in woodland plots of 5,445 square feet (⅛ acre), and those against *splendens* in plots of 2,500 square feet along the grassy margins of lakes.

Chigger abundance was determined by collecting them

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[2] A continuation of investigations formerly supported by funds allotted by the Department of the Army.

[3] Some of the experiments were conducted by C. C. Deonier, J. B. Gahan, G. C. Labreeque, H. C. Chapman, A. N. Davis, and T. Henneberry.

on 6-inch squares of black oilcloth or plastic placed in 20 favorable locations in each plot, such as stumps, logs, tufts of grass, and mouse runs. After 1 minute the oilcloth or plastic was removed and the chiggers were counted and returned to the plot. The effectiveness of a treatment was based on the differences in chigger counts before and at various intervals after treatment, adjusted for variations in the check plots by a modification of Abbott's formula.

Against *alfreddugesi* sprays were applied with a compressed-air sprayer, most of them at the rate of 24 gallons

Table 1.—Control of *Eutrombicula alfreddugesi* with emulsions and dusts of various acaricides. (3 to 11 replications.)

Acaricide and Formulation	Pounds of Toxicant per Acre	Per Cent Reduction at Indicated Weeks after Treatment					
		1–2	3–4	5–6	7–8	9–10	11
Toxaphene Em	1	85	83	80	72	74	—
	2	95	94	93	92	—	95[a]
Toxaphene D	5	98	93	96	91	—	—
Dieldrin Em	0.5	96	92	77	73	—	—
	1	98	95	84	87	91	94[b]
	2	100	99	—	94	—	—
Parathion Em	1	87	83	—	—	—	—
	2	97	97	89	78	—	—
Parathion D	1	95	90	88	93	82	53
	2	93	90	87	57	—	—
BHC (about 12% gamma) Em	1	80	85	75	79	—	—
	2	99	92	84	80	—	—
	3	—	97[c]	—	92[d]	—	—
BHC (5% gamma) D	2	92	81	82	83	—	—
Chlordane Em	1	80	81	75	69	—	—
	2	92	87	93	89	—	—
	3	—	92[c]	—	87[d]	—	—
Chlordane D	2	97	90	80	76	—	—
3,5-Dinitro-o-cresol Em	2	96	93	87	32	—	—
4,6-Dinitro-o-*sec*-butylphenol Em	2	97	92	—	—	—	—
Chlorthion Em	0.5	90	34	—	0[d]	—	—
	1	97	87	—	75[d]	—	—
	2	95	97	—	86[d]	—	—
Diazinon Em[e]	0.5	90	32	—	73[d]	—	—
	1	91	75	—	73[d]	—	—
	2	97	92	—	0	—	—
Malathion Em	0.5	91	61	—	0[d]	—	—
	1	93	62	—	32[d]	—	—
	2	96	59	—	16[d]	—	—
Hydroxypentamethylflavan Em	2	93	83	—	—	—	—
Chloroethyl chlorobenzenesulfonate Em	2	77	—	—	—	—	—
Octyl thiocyanate Em	2	76	—	—	—	—	—
DMC Em	1	75	62	41	—	—	—
Isobornyl thiocyanoacetate Em	2	68	—	—	—	—	—
Dodecyl thiocyanate Em	2	57	—	—	—	—	—
Dithiocyanodiethyl ether Em	2	51	—	—	—	—	—
Phenoxathiin Em	2	51	—	—	—	—	—
Hydroxycaprophenone Em	2	35	—	—	—	—	—
Dimethylaminophenyl thiocyanate Em	2	20	—	—	—	—	—

[a] 95% and 82% after 14 and 17 weeks, respectively.
[b] 88% and 83% after 14 weeks and 16 weeks, respectively.
[c] Observations on 4th week only.
[d] Observations on 8th week only.
[e] 100 gallons of spray per acre.

Table 2.—Control of *Trombicula splendens* with various acaricides.[a]

Acaricide and Pounds per Acre	Per Cent Reduction after—								
	24 Hrs.	48 Hrs.	1 Wk.	2 Wks.	4 Wks.	8 Wks.	10 Wks.	14 Wks.	16 Wks.
Endrin									
0.1	—	96	99	93	88	5	29	—	—
.2	—	97	98	83	95	98	89	76	35
.5	—	99	99	99	99	97	99	91	84
1.0	—	100	100	100	99	99	99	99	91
Dieldrin[b]									
0.1	87	—	77	74	27	—	—	—	—
.25	82	—	49	43	36	—	—	—	—
.5	97	—	98	94	89	—	—	—	—
1.0	99	—	98	97	90	—	—	—	—
Dieldrin									
0.1	78	86	57	18	19	—	—	—	—
.2	95	98	93	60	15	—	—	—	—
.5	98	91	94	80	79	89	11	—	—
.75	—	—	94	95	71	—	—	—	—
1.0	—	99	96	81	94	92	0	—	—
Lindane									
0.1	68	58	44	29	0	—	—	—	—
.2	88	87	45	38	47	—	—	—	—
.5	96	100	92	85	74	—	—	—	—
1.0	100	99	97	93	92	—	—	—	—
Isodrin									
0.2	—	93	81	51	11	—	—	—	—
.5	—	96	95	79	44	72	58	—	—
1.0	—	99	89	93	45	77	56	32	0
2.0	—	98	99	93	72	89	94	53	3
BHC (gamma)[c]									
0.1	85	83	45	33	—	—	—	—	—
.2	85	81	68	35	2	—	—	—	—
.5	96	90	72	54	7	—	—	—	—
Heptachlor									
0.1	68	33	28	16	6	—	—	—	—
.2	86	77	46	21	0	—	—	—	—
.5	97	94	80	74	28	—	—	—	—
1.0	95	98	71	26	68	—	—	—	—
Chlordane									
1.0	82	—	70	25	—	—	—	—	—
2.0	90	—	76	65	—	—	—	—	—
Dilan									
0.1	83	52	54	51	16	—	—	—	—
.2	90	94	72	25	—	—	—	—	—
.5	32	46	30	23	—	—	—	—	—
1.0	30	52	19	0	—	—	—	—	—
2.0	51	46	28	41	35	—	—	—	—
Aldrin									
0.2	77	65	57	26	0	—	—	—	—
.5	76	73	53	20	1	—	—	—	—
1.0	60	65	55	50	0	0	—	—	—
2.0	—	89	80	56	30	0	—	—	—
TEPP									
0.1	33	—	8	0	—	—	—	—	—
.25	74	—	30	29	—	—	—	—	—
.5	58	—	40	56	—	—	—	—	—
1.0	58	—	33	7	—	—	—	—	—
Parathion									
0.1	3	—	0	0	—	—	—	—	—
.25	32	—	11	16	—	—	—	—	—
.5	55	—	30	52	—	—	—	—	—
1.0	48	—	47	64	—	—	—	—	—
EPN									
0.1	36	—	21	19	—	—	—	—	—
.25	51	—	36	33	33	—	—	—	—
.5	38	—	13	64	0	—	—	—	—
1.0	36	—	20	18	0	—	—	—	—
NPD									
0.1	18	—	0	0	—	—	—	—	—
.25	64	—	46	61	22	—	—	—	—
.5	79	—	0	37	0	—	—	—	—
1.0	12	—	20	27	0	—	—	—	—
Aramite[b]									
0.05	0	—	—	—	—	—	—	—	—
.1	57	—	49	59	80	—	—	—	—
.25	51	—	55	59	64	—	—	—	—
.5	45	—	37	33	44	—	—	—	—

[a] Two to seven replications; emulsions unless otherwise noted.
[b] Wettable powder.
[c] From technical BHC containing 36% of gamma isomer.

per acre, and the dusts with a rotary hand duster at 10 to 40 pounds per acre. Against *splendens* sprays were applied with a power sprayer at the rate of 100 gallons per acre. Results of tests against *alfreddugesi* are summarized in table 1 and those against *splendens* in table 2.

Against *alfreddugesi* toxaphene in an emulsion was highly effective for 14 weeks at 2 pounds of toxicant per acre and gave good control for 5 to 6 weeks at 1 pound. Toxaphene in a dust at 5 pounds of toxicant per acre was highly effective for at least 8 weeks, when the tests were terminated. Dieldrin gave excellent control for 11 weeks at 1 pound per acre, for more than 8 weeks at 2 pounds, and for 3 to 4 weeks at ½ pound. The other acaricides were less effective.

Against *splendens* endrin gave excellent control for 16 weeks at 1 pound per acre and for 14 weeks at ½ pound. Dieldrin emulsion at 1 pound of insecticide per acre gave a high degree of control for 8 weeks; dieldrin wettable powder and lindane emulsion at 1 pound were highly effective through 4 weeks, when observations were discontinued. BHC (36% gamma) was about equal to lindane. The other acaricides were less effective.

REFERENCES CITED

Linduska, J. P., F. A. Morton, and W. C. McDuffie. 1948. Tests of materials for the control of chiggers on the ground. Jour. Econ. Ent. **41**(1): 43–7.

Smith, C. N., and H. K. Gouck. 1947. The control of chiggers in woodland plots. Jour. Econ. Ent. **40**(6): 790–5.

Traub, R., H. D. Newson, B. C. Walton, and J. R. Andy. 1954. Efficacy of dieldrin and aldrin in area control of the chigger vectors of scrub typhus. Jour. Econ. Ent. **47**(3): 429–35.

Control of Soil Insects on Turnips[1]

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Experiments for the control of soil-inhabiting insects attacking turnips were conducted in southern California from 1954 to 1956. The principal insects were the seed-corn maggot, *Hylemya cilicrura* (Rond.), the sugar-beet wireworm, *Limonius californicus* Mann., and occasionally white grubs. Wireworms and white grubs attack this crop during the spring and early summer, and the seed-corn maggot is destructive during the entire year. (Figures 1 to 4).

Morrison & Crowell (1953), in comprehensive studies of various insecticides against soil insects in Oregon, found that aldrin, dieldrin, and possibly heptachlor met the requirements for all-purpose insecticides in that State. Stitt (1953) reported that aldrin and heptachlor dusts applied in the furrow with the turnip seed gave satisfactory control of maggots, principally the cabbage maggot, and were superior to dieldrin, endrin, chlordane, and isodrin. He reported inferior results with surface applications. King & Forbes (1954), using the "band method" for control of root maggots on rutabagas, found that aldrin and heptachlor were the most effective and were superior to dieldrin.

FIG. 1.—Turnip showing holes and feeding scars made by larva of the seed-corn maggot.

GENERAL PROCEDURE.—Seven experiments were conducted in commercial fields of purple-top turnips near Norwalk. The plots were arranged in randomized blocks, with 4 to 6 replicates of each treatment. The plots varied in width from 14 to 40 feet and were 100 feet long. The insecticides were applied as emulsions to the soil surface with a power sprayer at the rate of 35 gallons per acre, from 1 to 21 days before planting. Only one application was made. Immediately after application the materials were thoroughly mixed with the soil, usually by disking 6 to 7 inches deep and by dragging to conserve soil moisture. Their effectiveness was determined by pulling at random 20 mature turnips per plot each week during the harvest period. After washing they were checked for injury. Turnips with shallow, unhealed feeding scars or with one or more holes were classified as being injured and unmarketable.

RESULTS.—The results of these experiments are presented in table 1. Approximately 80% of the damage was due to the seed-corn maggot and 10% each to wireworms and white grubs.

In experiment 1 the materials were applied on May 14, 1954, and the turnips planted the same day. Examinations for insect injury were made between July 13 and 29. Dieldrin, endrin, and heptachlor gave good control, and dieldrin was significantly better than isodrin, aldrin, DDT, toxaphene, and the check.

In experiment 2 the insecticides were applied on August 3, 1954, and the turnips planted on August 9.

[1] Accepted for publication October 1, 1956.

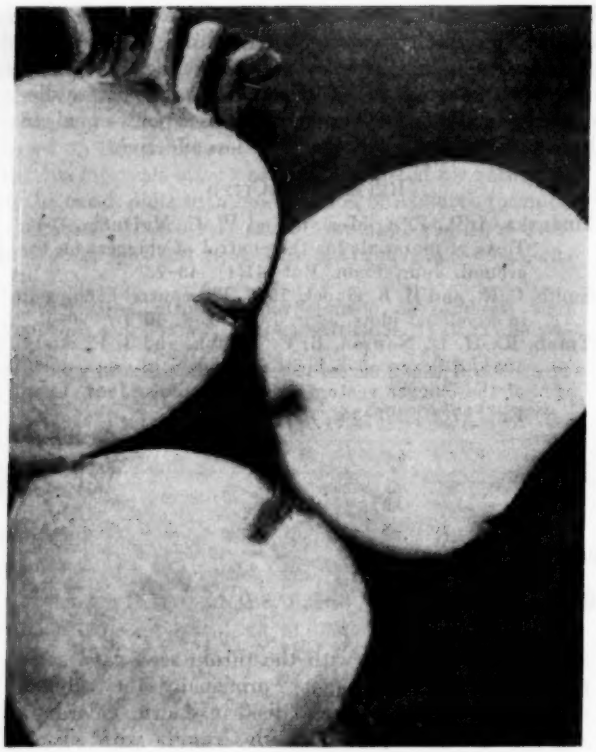

FIG. 2.—Half-sections of turnip showing burrows made by larva of the seed-corn maggot.

Examinations from October 4 to 25 showed that only dieldrin gave satisfactory control (less than 5% injury).

In experiment 3, in which three of the most promising materials were compared, none were highly effective because they had been mixed with the soil by plowing instead of by disking, but dieldrin was superior to endrin

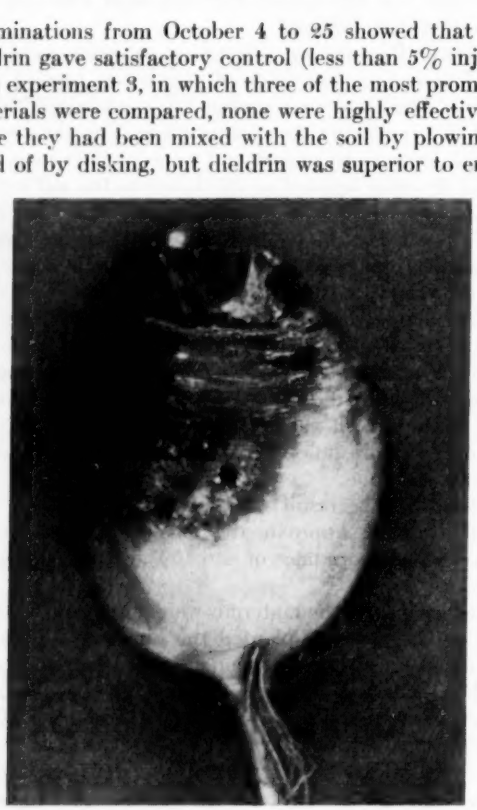

FIG. 3.—A turnip containing large holes made by the sugar-beet wireworm.

FIG. 4.—Scars on turnips due to the feeding of white grubs.

Table 1.—Control of the seed-corn maggot and sugar-beet wireworm on turnips with various insecticides. Norwalk, Calif., 1954–56.

Insecticide[a]	Pounds per Acre	Turnips Damaged by Insect Feeding	
		Number	Per Cent
Experiment 1 (4 replicates, 200 turnips)			
Dieldrin	2.6	3	1.5
Endrin	2.6	6	3.0
Heptachlor	2.6	10	5.0
Isodrin	2.6	16	8.0
Aldrin	3.0	19	9.5
DDT	10.5	22	11.0
Toxaphene	10.5	22	11.0
Untreated	—	29	14.5
L.S.D. at 5% level		12.6	6.3
Experiment 2 (6 replicates, 360 turnips)			
Dieldrin	2.7	12	3.3
Heptachlor	2.5	19	5.3
Aldrin	2.5	22	6.1
Endrin	2.5	23	6.4
Isodrin	2.5	32	8.9
DDT	10.7	40	11.1
Toxaphene	10.0	52	14.4
Untreated	—	63	17.5
L.S.D. at 5% level		32	8.9
Experiment 3 (3 replicates, 240 turnips)			
Dieldrin	2.3	15	6.2
Endrin	3.0	28	11.7
Heptachlor	3.0	45	18.8
Untreated	—	56	23.3
L.S.D. at 5% level		15.6	6.5
Experiment 4 (6 replicates, 240 turnips)			
Dieldrin	3.2	2	0.8
Heptachlor	3.3	4	1.7
Aldrin	4.2	4	1.6
Isodrin	3.2	5	2.1
DDT	18.0	9	3.7
Toxaphene	19.0	10	4.2
Endrin	3.2	12	5.0
DDT	9.0	17	7.1
Untreated	—	26	10.8
L.S.D. at 5% level		10.3	4.3
Experiment 5 (6 replicates, 360 turnips)			
Dieldrin (granulated)	3.5	6	1.7
Aldrin	4.7	12	3.3
Isodrin	3.3	13	3.6
Heptachlor	3.4	14	3.9
Endrin	3.3	15	4.2
Dieldrin	3.3	17	4.7
Heptachlor (granulated)	3.5	18	5.0
Untreated	—	20	5.6
L.S.D. at 5% level		—[b]	—[b]

Insecticide[a]	Pounds per Acre	Turnips Damaged by Insect Feeding	
		Number	Per Cent
Experiment 6 (6 replicates, 480 turnips)			
Heptachlor	3.2	10	2.1
Endrin	3.5	10	2.1
Dieldrin	3.3	11	2.3
Aldrin	3.9	17	3.5
Toxaphene	16.0	29	6.0
DDT	15.0	34	7.1
Untreated	—	111	23.1
L.S.D. at 5% level		17.8	3.7
Experiment 7 (6 replicates, 480 turnips			
Dieldrin	3.2 (17.5)[c]	3	0.6
	3.0 (32)	4	0.8
	3.2 (62)	5	1.0
Endrin	3.1 (17.5)	11	2.3
	3.0 (32)	14	2.9
	3.2 (62)	20	4.2
Untreated	—	76	15.8
L.S.D. at 5% level		26.9	5.6

[a] Emulsions unless otherwise indicated.
[b] Differences not significant according to *F* test.
[c] Figures in parenthesis indicate number of gallons of spray used.

and heptachlor even though they were applied at slightly higher rates. In this experiment the applications were made on August 17, 1954. The turnips were planted on August 24 following an irrigation, and harvested between October 14 and November 1.

In experiment 4 eight insecticides were applied on November 18, 1954. The turnips were planted on November 19, harvested between March 15 and 23, 1955. Dieldrin, heptachlor, and aldrin gave the best control (less than 2% damage). There was no significant difference between any of the materials used, except that DDT at 9 pounds per acre was not effective. During the winter months only 11% of the turnips in the untreated plots were damaged as compared with 15 to 23% during the previous summer and fall. In November 1955, after a crop of table beets had been grown in the summer, the entire experimental site was replanted to turnips. On April 25, 1956, 1½ years after the initial treatment, 40 turnips per plot were inspected for insect damage. Dieldrin, with 10.8% of the turnips damaged, continued to give the best control of all soil pests. Heptachlor and isodrin, with 12.1 and 12.9% damaged, ranked next, but none of the materials gave commercial control after this length of time, as the turnips in plots treated with aldrin, endrin, toxaphene, and both dosages of DDT had damage ranging from 17.1 to 30%.

In experiment 5 granulated formulations of heptachlor and dieldrin were compared with various insecticides in emulsions. To distribute the granulated insecticides evenly, the required amounts were mixed with 10 pounds of sand and broadcast over the soil surface. All materials were applied on March 21, 1955. The entire field was irrigated on April 4 and planted to turnips on April 10. Data on injury were obtained in the period June 13 to July 5. Insect populations in this field were too low for a proper evaluation of these materials. Granulated dieldrin showed the greatest promise.

In experiment 6 the insecticide applications were made on November 16, the turnips were planted on November 17, 1955, and harvested on February 20 and March 13, 1956. Heptachlor, endrin, dieldrin, and aldrin gave satisfactory control of all soil pests, with not more than 3.5% of the turnips damaged. Toxaphene and DDT with 6 and 7% damage, did not give commercial control (5% damage or less).

In experiment 7 various quantities of spray containing dieldrin and endrin applied at similar rates per acre were compared. The treatments were made on November 8, 1955, and the turnips planted the same day. Infestation counts were made between February 6 and March 12, 1956. There were no significant differences in the effectiveness of the different gallonages of either dieldrin or endrin spray, but all strengths and materials were superior to the check. With both materials the lower gallonage gave slightly better control, and at all strengths dieldrin was more effective than endrin.

SUMMARY.—From 1954 to 1956 seven insecticides were tested for the control of the seed-corn maggot, *Hylemya ciliicrura* (Rond.), the sugar-beet wireworm, *Limonius californicus* Mann., and white grubs in commercial plantings of turnips in southern California. Dieldrin at 2.3 to 3.3 pounds per acre gave the best and most consistent control for one season and partial control for 18 months. Heptachlor and endrin at similar dosages, and aldrin at slightly higher amounts, gave only commercial control (less than 5% of the turnips showing injury) for one season. Isodrin, toxaphene, and DDT at 9 to 11 pounds per acre did not give adequate protection for this crop.

REFERENCES CITED

King, K. M., and A. R. Forbes 1954. Control of root maggots in rutabagas. Jour. Econ. Ent. 47(4): 607–15.

Morrison, H. E., and H. H. Crowell 1954. Soil insecticide studies in Oregon. Jour. Econ. Ent. 45(6): 1002–10.

Stitt, Loyd L. 1953. Insecticide tests for control of maggots in turnips. Jour. Econ. Ent. 46(6): 961–5.

Studies on Rearing the House Fly on a Bran-Alfalfa Medium[1]

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When the Pesticide Testing Laboratory was recently established, it was decided to set up facilities for rearing the house fly, *Musca domestica* L., and to use it as a test insect in the Peet-Grady chamber. The Peet-Grady Method, as reported in the annual editions of Soap and Chemical Specialities Blue Book, was used as a guide, and scientists employing this technique were frequently consulted. In the process of becoming familiar with the technique, many variations of the rearing method were tested. The results of some of these investigations are reported here.

The literature reveals that house flies have been reared year round on a variety of materials from a natural medium of horse manure with commercial bakers' yeast (Glaser 1927) to the artificial and now popular bran-alfalfa mixture of Richardson (1932). Some workers have reared flies on common bacteriological media (Gerberich 1948) and others on known bacterial flora (Silverman & Silverman 1953). Hafez (1949) presented a review of various media used by workers as diets for breeding the house fly under laboratory conditions.

The formula for the fermenting medium on which our house fly maggots[3] were being reared at the time of these studies was:

bran-alfalfa meal (2:1)		382 gm.
water	approximately	800 ml.
yeast		7.50 gm.
diastatic malt extract		6.25 gm.

At the conclusion of the experiments recorded here, this laboratory adopted the Chemical Specialties Manufacturers Association (C.S.M.A.) larval medium as described in the Soap and Chemical Specialities Blue Book 1956 as its standard larval diet for house flies.

The size and number of pupae produced from a measured quantity of medium is largely a function of the interaction of (1) amount of dry ingredients, (2) amount of water, and (3) number of maggots. A change in the proportion of one of these necessitates a compensating change in the proportions of the other two. In our investigation of this interaction, we set the weight of dry ingredients at 382 gm. as a basic starting point. We then adjusted amount of water and number of maggots. Our observations on these major factors, as well as on other factors of lesser importance, are reported.

Cultures were held in glass battery jars having an inside diameter of 6 inches and a height of 8 inches. Medium was mixed in a Hobart N-50 cake mixer having a capacity of 5 U. S. quarts, sufficient to mix medium for one jar at a time. The rearing room was held at $28 \pm 1°$ C. and $50 \pm 5\%$ R.H.

Dry Ingredients.—Richardson (1932) used a mixture of wheat bran and alfalfa meal in the ratio of 2:1 by weight. The Peet-Grady Method (Anonymous 1956) recommends the use of the C.S.M.A. standard dry larval medium composed of alfalfa meal, dried brewers' grains, and soft-wheat bran in equal amounts by weight. In a personal communication, Goddin (1953) stated that finely ground alfalfa meal that has been sun-dried should be used and that the wheat bran should be coarse. The brewers' grains provide bulk and permit air exchange in the fermenting medium but are not a necessity if the bran and alfalfa meal are not too fine in particle size. Basden (1947) used two parts by weight of middlings to one part of grass meal. Bergmann & Levinson (1954) omitted the alfalfa meal, while Wolfinsohn (1951) used straw and bran in the ratio of 1:1 by weight. Eagleson (1937) found success using fermenting crimped oats. David (1946) replaced the alfalfa meal with grass meal and dried blood. Wilkes *et al.* (1948) found "that the larvae could be reared in a medium in which all or part of the alfalfa meal was replaced by sawdust," that "sawdust could not be substituted for any part of the bran," and that the use of sawdust in place of alfalfa "had a marked effect on the production of flies. The yield of puparia was considerably lower and there was a noticeable reduction in emergence of adults from the puparia."

The larval medium used in most of these experiments was the bran-alfalfa formula recorded above. However, experiments were performed in June 1955 and in February, March, and May of 1956 to compare our locally prepared bran-alfalfa with the C.S.M.A. standard bran-alfalfa brewers' grains mixture. Equal quantities of yeast, malt, water, and house fly eggs were added to each jar, and the C.S.M.A. standard medium was from a single lot received in March 1955. Table 1 gives the averages for these four tests as well as the averages from the routine rearing of large numbers of flies during the same periods.

It will be noted that with the locally mixed medium the yields in grams and the numbers of pupae[4] per gram were uniform in all periods reported. The variability in results with the C.S.M.A. standard medium cannot be explained satisfactorily. In February, March, and May, the C.S.M.A. averages show a smaller yield and heavier pupae (fewer per gram) than the locally mixed medium. The routine rearings in June 1956 showed an improvement in production, but we are unable to explain the excellent yield of heavy pupae obtained in June 1955 under conditions that were, as far as we could determine, identical with those in all the other rearings.

Some of the flies from cultures of June 1955 and May 1956 were tested in the Peet-Grady chamber with the Official Test Insecticide and failed to demonstrate any significant difference in mortalities attributable to the larval diets employed here.

Amount of Water.—Basden (1947) recognized the importance of getting the proper quantity of water to

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[3] The flies used in this study were from the National Association of Insecticide and Disinfectant Manufacturers strain, received in Canada in 1948 and bred continuously since that date.

[4] Whenever pupae were weighed, measured, or counted, they were invariably enclosed in puparia.

Table 1.—Average yield of house fly pupae and average pupae per gram from two different media at various periods.

DATE	LOCALLY MIXED MEDIUM				C.S.M.A. STANDARD MEDIUM			
	No. of Jars	Yield (Gm.)	Pupae per Gram	Calculated Total[a]	No. of Jars	Yield (Gm.)	Puape per Gram	Calculated Total
				Investigational				
June 1955	12	43	48.6	2090	8	47	45.6	2143
February 1956	6	42	48.7	2045	6	32	45.9	1469
March 1956	11	42	48.2	2024	11	32	46.4	1485
May 1956	12	40	48.3	1932	12	29	46.9	1360
				Routine rearing				
June 1955	66	43	48.4	2081				
February 1956	65	43	47.8	2055				
June 1956					54	38	48.5	1843

[a] The product of yield and pupae per gram.

ensure obtaining insects of a uniformly large size. Wilkes *et al.* (1948) stated, "If the medium became too wet, aeration was poor and fermentation dropped, whereas if the medium became dry aeration was excessive and fermentation proceeded too rapidly." Wolfinsohn (1951) reared flies on media of different moisture contents and showed that there is an optimum water level.

Our observations merely corroborated the findings of others. When too much water is added, free water floods the bottom layer of the medium and anaerobic putrefaction starts there. To avoid drowning, the maggots congregate in the upper layers where crowding and limitation of food supply result in production of small pupae. When insufficient water is added, a gray, ashy, central core is formed. This is apparently caused by excessive heat of fermentation and again results in the production of small pupae. Mold is frequently present, and the surface of the medium is usually covered with a hard, dry crust at the time of pupation. When water level is properly adjusted, no free water can be seen at the bottom of the medium on the fifth day; at the time of complete pupation, the medium is covered with a layer of dark-brown, dry, flaky material. The color is uniformly brown for the most part, shading into a yellowish brown in the very lowest portion.

In our laboratory it is customary to check water level once a month, and also when a new shipment of medium is introduced, by adding an extra 50 ml. of water. If this leaves a quarter of an inch of free water in the jar, we are assured that our standard amount is correct.

YEAST AND MALT.—Many authors, starting with Richardson (1932), have reported on rearing house flies on the bran-alfalfa type of medium. Richardson suspended bakers' yeast in water, stored it on ice, and measured out a portion into each mixture of medium. Presumably his suspensions were used up quickly. Basden (1947) used dried active distillers' yeast and stated that a dried inactive brewers' yeast also gave excellent results. Most authors (Eagleson 1937, Wilkes *et al.* 1948, Harrison 1949, and others) used paste or bakers' yeast, apparently mixing it with water as needed. Wilkes *et al.* (1948) found that yeast could be kept in cake form for not longer than 10 days after purchase.

The idea of using a stock suspension of yeast and a solution of malt extract, stored under refrigeration at about 5° to 6° C., was attractive from the point of view of convenience, and it was decided to investigate the keeping quality of the yeast. A suspension of 1 pound of fresh bakers' yeast in an equal weight of aged tap water was prepared. Samples from this suspension were tested by the Division of Bacteriology and Dairy Research twice weekly for a period of 6 months in one test and 8 months in another, both tests involving duplicate samples. In both investigations the yeast counts were initially between 4 and 6 billion per c.c. and remained at approximately 4 billion for 105 days. Thereafter, the counts fell gradually to about 1 to 2 billion at 180 to 200 days. It was concluded that yeast suspension prepared and stored in this manner would be useful for about 4 months. This is more than enough, since a pound of yeast lasts less than 1 month in our laboratory.

Our malt extract is also prepared as a stock solution, using hot water for easier dissolving, and is stored in the refrigerator. Our formula for dilution is stated in terms of weight, since we find it easier to weigh than to measure the malt by volume as suggested by The Peet-Grady Method (Anonymous 1956). For a comparison of volume and weight, it is probably sufficient to calculate on the basis of a specific gravity of about 1.33. Occasionally a mold will appear and the flask is then discarded, but losses are insignificant.

Although Wilkes *et al.* (1948) stated, ". . . varying the concentrations of the different ingredients composing the rearing medium had little effect on the production of puparia," it was decided to investigate the importance of malt and yeast for our own satisfaction. In these experiments, bran-alfalfa at 382 gm. and water at 800 ml. were kept constant, and 0.3 ml. of eggs (pipette method, Moreland & McLeod 1956) were added to each jar.

In the first experiment, malt and yeast were added in equal proportions at the following levels: 15, 7.5, 3.8, and 1.9 gm. These four diets were replicated on 8 different days. The analyses of variance on yield in grams and average number of pupae per gram showed no significant difference between diets. The difference between replicates was greater than the difference between diets.

In the second experiment, the yeast was constant at 7.5 gm. per jar, while malt was added at the rate of 12.5, 6.3, 3.1, and 1.6 gm., replicated 10 times. Once again the analyses of variance of both yield in grams and average

Table 2.—Pupal yield from bran-alfalfa medium with neither malt nor yeast.

Jar No	Pupae		
	Yield (Gm.)	No. per Gram	Calculated Total
1	17.4	78.6	1368
2	15.8	70.6	1115
3	9.3	86.6	805
4	14.4	65.2	939
Average	14.2	75.3	1057
Average of 42 jars using ordinary medium	36.0±4.2	50.7±2.4	1816±201

number of pupae per gram showed no significant difference between diets, and the difference between replicates was greater than between diets.

Finally, jars were seeded that had bran-alfalfa and water only, with no yeast or malt. Results were variable, as shown in table 2. No effort was made in this experiment to sterilize mixing or handling equipment or to prevent the invasion of air-borne spores. Neither was any attempt made to determine why the pupae were fewer in number or lighter in average weight in the absence of yeast and malt.

When medium without yeast and malt was allowed to stand for 24 hours before eggs were added, molds became established and over 90% of the maggots died before pupating.

It was concluded that, while flies can be reared on bran-alfalfa and water, the addition of yeast and malt assures a satisfactory start of the fermenting process and gives a reasonable uniformity in production of pupae. The evidence indicates that amounts of yeast and malt are of no significance within the limits tested.

Effect of Various Seeding Rates on Yield and Size of Pupae.—It is an established fact that cultures seeded with large numbers of eggs produce small flies (Thomssen & Doner 1938, Smith & Harrison 1951, and Wilkes *et al.* 1948). Studies were made to show relative numbers of pupae in size groups from different seeding rates. At the same time, the calculated total, obtained by multiplying the yield in grams by the number of pupae per gram, was checked against actual counts of total numbers. Emergence and sex ratios were also recorded.

Eggs were seeded by the pipette-tube method (Moreland & McLeod 1956) at the following rates: 0.1, 0.2, 0.3, 0.5, and 0.7 ml. Eight days later the pupae were weighed, and the average number of pupae per gram was determined by counting 5-gm. samples. The pupae were then sieved through slotted screens having 7.0, 6.5, 6.0, and 5.5 sixty-fourths inch slot widths. Those from each sieve were counted and totals were checked against calculated totals (table 3). It will be seen that calculated totals are reasonably accurate.

Table 4.—Emergence and sex ratios from pupae sized by slotted screens.

Screen Slot Widths (1/64 In.)	Pupae per Gram	Emergence (Per Cent)	Sex Ratio	
			Male (Per Cent)	Female (Per Cent)
<5.5	106	32.8	71	29
5.5	70	59.6	68	32
6	54	78.8	54	46
6.5	44	87.6	33	67

At the lower seeding rates, mold was noticeable, and a fairly heavy crust and a gray, ashy, central core were observed. However, the puparia were thoroughly darkened, and few maggots were seen on the eighth day after seeding. At the higher seeding rates, no mold, crust, or core were found; but development of the larvae was delayed. Many had not pupated on the eighth day, and existing puparia were freshly formed and pale in color.

Pupae graded according to size were placed in rearing to determine percentage emergence and sex ratio (table 4). Per cent emergence increased as size increased. The smaller pupae were predominantly male, whereas the larger ones were mostly females.

Overnight Eggs.—Some authors (Harrison 1949, Wilkes *et al.* 1948, Anonymous 1956) seeded eggs into medium that had been mixed the day before, while others seeded freshly prepared medium. There was also variation between ages of eggs used. The Peet-Grady Method (Anonymous 1956) states that eggs should be collected for a period of not longer than 16 hours.

At the Pesticide Testing Laboratory, oviposition pads are left in the stock cage from 9 A.M. to 2 P.M., and the eggs are placed in medium mixed the same day. Pupae are separated from the medium on the eighth day, and first emergence of adults is observed on the 10th day. Peak emergence occurs during the 11th day, and first oviposition occurs occasionally on the 13th day but more commonly on the 14th day.

Observations were made on the development of eggs laid during a 16-hour overnight period. A very few maggots were noted at 9 A.M. At this hour the eggs were washed thoroughly in three changes of water. Floating eggs were discarded. A sample of the submerged eggs was set out on moist black cotton at 28° C. The average hatch had risen to 7% by 3 P.M. and was 94.8% by the following morning. This compares favorably with a long-time

Table 3.—Numbers and sizes of house fly pupae retained on slotted screens from various seeding rates.

Seeding Rate (Ml.)	Screen Slot Widths (1/64 In.)					Total No.	Pupae		
	7	6.5	6	5.5	<5.5		Yield (Gm.)	No. per Gm.	Calculated Total
0.1	11	495	255	56	20	837	19.4	43.8	850
.2	3	276	763	467	59	1568	31.0	52.0	1612
.3	0	582	1084	375	50	2091	43.3	50.0	2165
.5	0	19	667	1896	375	2957	51.0	60.8	3101
.7	0	0	114	2101	1525	3740	52.7	71.4	3763

average hatching rate of 91.5% of eggs laid between 9 A.M. and 2 P.M. and similarly washed and selected.

When overnight eggs are seeded in the morning on freshly mixed medium, they develop at approximately the same rate as the regular cultures seeded during the afternoon of the same day. On the other hand, when overnight eggs are seeded on medium mixed the previous day and allowed to ferment for 18 hours, the rate of development is comparable to that found in cultures seeded the previous afternoon on fresh medium. One may take advantage of this in mass production of flies of uniform age on the day of testing. Occasionally an inadequate supply of eggs is obtained during the normal 9 A.M. to 2 P.M. period. Medium is prepared as usual, is held over night, and is seeded at 9 A.M. the following morning with overnight eggs. The resulting adults are as suitable for bioassay of insecticides as those produced according to the standard rearing schedule.

REPRODUCIBILITY.—Wilkes *et al.* (1948) stated that the most striking variable encountered in the mass production of house flies was the number of puparia per jar of rearing medium.

An attempt was made to obtain reproducible results. Three jars of medium were seeded daily for 4 consecutive days, using eggs from the same stock cage and measuring them with a single pipette tube (Moreland & McLeod 1956). Separation, cleaning and weighing of pupae were done by one worker. Results from 12 jars were: yield, 41.6 ± 3.1 grams per jar; pupae per gram, 50.4 ± 1.9; and calculated total, 2096 ± 184 pupae per jar.

The standard deviations of these values may be compared with the following statistics based on 30 months' continuous rearing, being a total of 1562 jars: yield, 38.9 ± 4.4 grams per jar; pupae per gram, 48.1 ± 2.0; and calculated total, 1838 ± 220 pupae per jar. It would seem reasonable to conclude that these standard deviations, resulting from a carefully conducted rearing program, represent more or less the limits of reproducibility of weight and numbers of pupae per jar with the rearing technique under discussion in this paper.

Fisher & Morrison (1949) and Fisher (1952) used pupae that averaged 22 mg., while Wilkes *et al.* (1948) recorded a mean weight of 19.5 ± 1.5 mg. The average weight per pupa in our laboratory was 20.8 mg. with a standard deviation of ± 0.9 mg.

SUMMARY.—House flies were reared on a bran-alfalfa medium, and the dry and wet ingredients were varied. The resulting pupal yields in grams and the numbers of pupae per gram were noted. Although there were unexplained variations in pupal production between the C.S.M.A. standard and locally mixed media, these variations did not show up in the Peet-Grady chamber. It was found that the amount of water in the medium was important for the production of reproducible numbers and weights of pupae. The amounts of yeast and malt used in the medium were of no significance within the limits tested. In the absence of yeast and malt, however, yield was reduced and number of pupae per gram was increased. Stock solutions of both yeast and malt may be stored in a refrigerator at 5° to 6° C. and used over a 4-month period.

House fly eggs were seeded at various rates, and the numbers of pupae in different size groups were recorded. It was shown that there was a close agreement between the calculated total of pupae per jar of rearing medium, obtained by multiplying the yield in grams by the number of pupae per gram, and the actual numbers of pupae per jar. There was an increase in percentage emergence as the size of pupae increased. The smaller pupae were predominantly male while the larger ones were mostly female.

Occasionally insufficient numbers of eggs are obtained during the normal oviposition period of 9 A.M. to 2 P.M. Eggs which have been oviposited throughout the following night may be seeded into fermented medium in order to prevent a lapse in the normal testing schedule. The resulting adults are as suitable for bioassay of insecticides as those produced according to the standard rearing schedule.

From 30 months' continuous rearing, representing 1562 jars of medium, the following averages and standard deviations were obtained: yield, 38.9 ± 4.4 grams per jar; pupae per gram, 48.1 ± 2.0; calculated total, 1838 ± 220 pupae per jar.

REFERENCES CITED

Anonymous. 1956. The Peet-Grady method. Soap and Chem. Spec. Blue Book pp. 243–4, 267.

Basden, E. B. 1947. Breeding the house-fly (*Musca domestica* L.) in the laboratory. I. Introduction and technique. Bull. Ent. Res. **37**: 381–7.

Bergmann, E. D., and Z. H. Levinson. 1954. Steroid requirements of housefly larvae. Nature **173**(4396): 211–2.

David, W. A. L. 1946. The quantity and distribution of spray collected by insects flying through insecticidal mists. Ann. Appl. Biol. **33**(2): 133–41.

Eagleson, C. W. 1937. Rearing house flies. Soap and Sanitary Chem. **13**(12): 115, 117.

Fisher, R. W. 1952. The importance of the locus of application on the effectiveness of DDT for the house fly, *Musca domestica* L. (Diptera: Muscidae). Canadian Jour. Zool. **30**: 254–66.

Fisher, R. S.[5] and F. O. Morrison. 1949. Methods of rearing and sexing (*Musca domestica* L.). Ann. Rept. Ent. Soc. Ontario **80**: 41–5.

Gerberich, J. B. 1948. Rearing house-flies on common bacteriological media. Jour. Econ. Ent. **41**(1): 125–6.

Glaser, R. W. 1927. Note on the continuous breeding of *Musca domestica*. Jour. Econ. Ent. **20**(2): 432–3.

Hafez, M. 1949. A simple method for breeding the house-fly, *Musca domestica*, L., in the laboratory. Bull. Ent. Res. **39**: 385–6.

Harrison, R. A. 1949. Laboratory breeding of the housefly, (*Musca domestica* L.). New Zealand Jour. Sci. and Technol. **30**(B4): 243–7.

Moreland, C. R., and W. S. McLeod. 1956. House fly egg-measuring techniques. Jour. Econ. Ent. **49**(1): 49–51.

Richardson, H. H. 1932. An efficient medium for rearing house flies throughout the year. Science **76**(1972): 350–1.

Silverman, P. H., and L. Silverman. 1953. Growth measurements on *Musca vicina* (Macq.) reared with a known bacterial flora. Trop. Dis. Bull. **50**(11): 1089–90.

Smith, A. G., and R. A. Harrison. 1951. Notes on laboratory breeding of the housefly (*Musca domestica* L.). New Zealand Jour. Sci. and Technol. **33**(B 1): 1–4.

Thomssen, E. G., and M. H. Doner. 1938. Breeding house-

[5] R. W. Fisher.

flies. A simplified and more convenient method of rearing and handling flies for Peet-Grady tests. Soap and Sanitary Chem. 14(10): 89–90, 101.

Wilkes, A., G. E. Bucher, J. W. MacB. Cameron and A. S. West, Jr. 1948. Studies on the house fly (*Musca domestica* L.). I. The biology and large scale production of laboratory populations. Canadian Jour. Res. D26: 8–25.

Wolfinsohn, M. 1951. The influence of the water content of the medium on the accumulation of larvae and pupae of the house-fly. Bull. Res. Counc. Israel 1(1–2): 166–8 (R. A. E. 41 (B 12): 199, 1953.).

The Relative Toxicity, Synergistic Activity, and Knockdown Effectiveness of Mixtures of Piperonyl Butoxide with Allethrin and Its *Trans* Fraction and Isomers as House Fly Sprays[1]

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With the preparation (LaForge *et al.* 1954) of the four *trans* stereoisomers of allethrin, which is a mixture of esters of *dl*-allethrolone with *dl-cis-* and *dl-trans*-chrysanthemumic acids, and the evaluation of their relative toxicities (Gersdorff & Mitlin 1953), questions arose regarding their joint action with a material known to synergize allethrin itself. The isomers were found by the Campbell turntable method to act similarly as space sprays against house flies, *Musca domestica* L., differing only in the degree of their action. Not only did probit mortality increase at the same rate as for allethrin with increase in log concentration, but mixtures of them, exhibiting neither synergism nor antagonism, were characterized as of the similar type of joint action as defined by Bliss (1939). Therefore, it is probable, if not certain, that a material would synergize each isomer and to the same degree, or very nearly the same, as it did allethrin and its *dl-trans* fraction. Then, equi-effective amounts of mixtures of the isomers with the synergist in the same proportion, when expressed as isomer equivalents, would exhibit the same toxicity relative to a like mixture of allethrin as the isomers alone exhibit to allethrin alone. When the amounts are expressed as allethrin equivalents, they would be the same as in the allethrin mixture and differ only from allethrin alone in proportion to the intensity of synergism involved. To see if these relationships could be demonstrated by experiment, a study was made in which the same testing procedure was used as in previous related studies and so was kept on a comparable basis with these.

The synergist used was piperonyl butoxide; its effects in allethrin mixtures were reported by Gersdorff & Mitlin (1951). Because of its low toxicity relative to that of allethrin, the determination of its ratio of toxicity was sufficiently precise for that study. Now, because of the low toxicity of the *ll-trans* isomer of allethrin, the estimation of that ratio has become of greater importance. Therefore, tests were repeated with the synergist and allethrin to obtain a more precise estimation of their relative toxicities.

As an additional check on the synergistic effects a study was also made with mixtures of the same synergist with the previously separated *cis* and *trans* fractions of allethrin, which have been bioassayed by the same procedure (Gersdorff & Mitlin 1952), and with a prepared mixture of the two.

It was likewise thought desirable to repeat tests with mixtures of this synergist with allethrin itself in both studies. Tests with a sample of pyrethrins known to be trustworthy were included as a check on the biological purity of the sample of allethrin.

MATERIALS.—The preparation and physical characteristics of the four stereoisomers are described by LaForge *et al.* (1954). In this paper they will be designated as the *l-d*, *d-l*, *d-d*, and *l-l trans*-allethrin, the first symbol in the pair referring to the optical activity of the allethrolone component of the molecule and the second to that of the acid component. The samples used were of a high degree of purity.

A distilled sample of allethrin, analyzing 95% by the hydrogenolysis method, was used in its mixtures and as the principal standard of comparison.

The same stock solutions of the *cis* and *trans* fractions of allethrin that were used in the bioassay of these fractions were used in this study. The mixture of them was prepared in the proportion in which they were found by the bioassay to occur in the sample of allethrin, that is, 69% *cis* and 31% *trans* isomers. Since the work on these fractions it has been found in the chemical studies that, although the *trans* fraction was authentic, serious doubts exist as to the purity of this sample of the *cis* fraction, and therefore as to its bioassay, at least some of it possibly converting to the *trans* form during its preparation. However, despite this, the sample will suffice as an additional check material of previously determined relative toxicity. Quotation marks will be used in connection with the material to avoid a confusion with future work on an authentic sample of the *cis* fraction. The *trans* fraction was also used alone as a confirmatory standard of comparison.

The sample of pyrethrins was one whose stability had been recently checked both chemically and biologically. Sixty-one per cent of its total pyrethrins consisted of pyrethrin I and cinerin I.

Piperonyl butoxide was selected as the adjunct because its intensity of synergism with allethrin had been previously evaluated by the same procedure and its solubility in refined kerosene was such that an auxiliary solvent, with its attendant complications, was not required. The sample used was the technical product obtainable commercially.

PROCEDURE.—Sprays were prepared by dissolving the

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materials in refined kerosene (Deobase) and, after preliminary tests, diluting with the same solvent to selected concentrations.

In estimations of relative toxicity at the median lethal concentration (LC-50), the slope of the regression lines affects the values but slightly if the concentrations of the sprays are so chosen as to cause probits of mortalities to fall in such a manner that the mean points are close to the LC-50's. Four concentrations for each material have usually sufficed in previous studies. However, it has been demonstrated in some studies of synergism that the regression line for the synergized mixture has a greater slope than the line for the insecticide alone. Therefore, to secure more information regarding parallelism of regression lines the number of concentrations was increased in the present work. To keep the number of tests made at one time against each population of insects within a reasonable limit, the sprays containing piperonyl butoxide were prepared only in the proportion of 10 times as much adjunct as isomer or isomeric mixture.

Knockdown in 25 minutes and mortality in 1 day were determined in tests with the sprays by the Campbell turntable method on house flies of the National Association of Insecticide and Disinfectant Manufacturers 1948 strain. The tests were made in several series, each series comprising the results obtained with the same populations of flies. Six or seven replications were made, all sprays in a series being tested simultaneously on the same population. The average age of flies in each series was 2 to 3 days.

To evaluate relative toxicity and determine the precision of the estimates, the mortality data were subjected to probit analysis as described by Finney (1952). The inclusion of concentrations of the piperonyl butoxide mixtures that caused very low mortality and consequently extended knockdown below the 50% level for most of the mixtures permitted for the first time by this method a statistical comparison of knockdown effectiveness. Therefore, the knockdown data for these mixtures were also subjected to probit analysis. Although, of course, knockdown is the result of a toxic action, in accordance with general usage the term "toxicity" refers only to the lethal action.

MORTALITY AND KNOCKDOWN.—The mortality and knockdown data are summarized in table 1. To permit a simple group classification of the isomers and their combinations, they are referred to as allethrinoids.

PARALLELISM OF REGRESSION LINES AND REQUIRED HETEROGENEITY FACTORS.—Provisional regression lines were fitted graphically to the mortality data in table 1. It appeared that the data for the piperonyl butoxide mixtures in series 1 could be adequately represented by parallel lines. In series 2 this also appeared to be true for the data for the mixtures, but the data for the *trans* fraction and allethrin themselves indicated that, although they could be represented by two parallel lines, their slope would be different from that of the mixtures. In series 3 it appeared that the data for allethrin and pyrethrins would be represented by lines of different slope, as is usual, but that the data for piperonyl butoxide would give a line of intermediate slope, which could perhaps be properly made parallel with either of the others. Because of the intimate relationship of piperonyl butoxide with allethrin in this study, it was deemed desirable to study the lines of these two for parallelism. Therefore, in these four cases of possible parallelism, the generalization procedure was followed to obtain the weighted regression equations. Individual equations were computed for allethrin in series 1 and pyrethrins in series 3.

For the study of knockdown effectiveness the data were likewise fitted by parallel lines for each group of piperonyl butoxide mixtures.

An analysis of chi-squares in each generalized group gave no evidence that the data could not be represented by parallel lines. For error estimations in the toxicity evaluations heterogeneity factors used were in series 1, 9.324 for the mixtures and 1 for allethrin; in series 2, 2.910 for the mixtures and 3.282 for the *trans* fraction and allethrin; and in series 3, 15.798 for piperonyl butoxide and allethrin and 4.123 for pyrethrins. In the evaluations for knockdown effectiveness, heterogeneity factors used were 8.225 in series 1 and 14.429 in series 2. The high factor for piperonyl butoxide and allethrin in series 3 was due solely to the contribution by the data for piperonyl butoxide. Comparatively high heterogeneity appears to be usual in data obtained by this method with materials of such very low toxicity as this and chemically similar synergists.

EVALUATION OF RELATIVE TOXICITY AND INTENSITY OF SYNERGISM.—The final equations showing the regression of mortality, expressed in probits, on concentration in milligrams per deciliter, expressed as logarithms, are given below. Those for the piperonyl butoxide mixtures are given on two bases, the expression of concentration in terms of allethrinoid equivalents (with constant 2) as well as in terms of allethrinoid content only (with constant 1). The standard errors (s_b) of the regression coefficients are also given.

	(1)	(2)	s_b
Series 1			
Piperonyl butoxide plus—			
l-d ester	$Y=4.6791X-2.0981$	-2.4393	0.178
d-l ester	$Y=4.6791X-4.4241$	-5.5822	.178
l-l ester	$Y=4.6791X-7.4416$	-10.9914	.178
d-d ester	$Y=4.6791X+1.0307$	$+0.9675$	.178
allethrin	$Y=4.6791X-1.3185$	-1.5236	.178
Allethrin	$Y=3.1127X-0.8398$	—	.091
Series 2			
Piperonyl butoxide plus—			
trans fraction	$Y=4.6324X-0.7799$	-0.9545	0.121
"*cis*" fraction	$Y=4.6324X-1.9385$	-2.1742	.121
"*cis*"-*trans* mixture	$Y=4.6324X-1.4684$	-1.6714	.121
allethrin	$Y=4.6324X-1.2372$	-1.4395	.121
trans Fraction	$Y=3.4907X-1.3065$	—	.146
Allethrin	$Y=3.4907X-1.5357$	—	.146
Series 3			
Piperonyl butoxide	$Y=3.4282X-8.5090$	—	0.290
Allethrin	$Y=3.4282X-1.6192$	—	.290
Pyrethrins	$Y=2.6740X-1.2125$	—	.203

From these equations LC-50's were estimated and relative toxicities were calculated as the inverse ratio of the relevant pair of estimations on the allethrinoid equivalent basis. They are reported in table 2. Since the extended nature of the first two series precluded the inclusion of tests with the allethrinoids and piperonyl butoxide separately, their relative toxicities as determined in other studies by this method (Gersdorff & Mitlin 1952, 1953) are used. However, this procedure is valid because of the close reproducibility of estimates of relative toxicity as defined by this method. Since there is frequent need for these estimates, they, with one exception, are also included in the table. Because the estimate for the *trans*

Table 1.—Mortality and knockdown of house flies caused by kerosene sprays selected to study the comparative effect of piperonyl butoxide in mixtures with allethrin isomers and certain combinations of them.

Material	Concentration (Mg. per Dl.)			Per Cent	
	Allethrinoid	Piperonyl Butoxide	Allethrinoid Equivalents[a]	Knockdown in 25 Minutes	Mortality in 1 Day
Series 1. Six replicates, 98 flies each					
Piperonyl butoxide plus *l-d* ester	66.67	666.7	78.87	100	91.5
	44.44	444.4	52.58	97.9	70.9
	29.63	296.3	35.05	97.1	36.6
	19.75	197.5	23.36	91.2	19.4
	13.17	131.7	15.58	65.3	2.3
	8.779	87.79	10.39	22.7	2.8
d-l ester	225.0	2,250	397.8	99.0	98.4
	150.0	1,500	265.2	92.8	81.5
	100.0	1,000	176.8	59.9	41.6
	66.67	666.7	117.9	42.4	15.8
	44.44	444.4	78.58	4.3	2.4
	29.63	296.3	52.39	2.2	2.0
l-l ester	1,139.0	11,390	6,533	49.4	99.2
	759.4	7,594	4,356	21.0	88.4
	506.3	5,063	2,904	6.1	48.5
	337.5	3,375	1,936	2.4	29.3
	225.0	2,250	1,291	0.4	7.7
	150.0	1,500	860.4	0.4	1.9
d-d ester	15.00	150.0	15.47	100	91.6
	10.00	100.0	10.31	99.9	78.6
	6.667	66.67	6.876	99.2	46.5
	4.444	44.44	4.584	97.2	11.5
	2.963	29.63	3.056	89.7	6.2
	1.975	19.75	2.037	45.2	1.5
allethrin	59.26	592.6	65.54	99.9	98.1
	39.51	395.1	43.70	99.4	91.0
	26.34	263.4	29.13	98.0	62.4
	17.56	175.6	19.42	92.4	31.8
	11.71	117.1	12.95	62.3	6.0
	7.804	78.04	8.631	26.3	1.0
Allethrin	200.0	—	200.0	100	89.2
	133.3	—	133.3	100	78.2
	88.89	—	88.89	100	62.9
	59.26	—	59.26	99.8	37.2
	39.51	—	39.51	99.0	17.0
	26.34	—	26.34	96.2	8.1
Series 2. Six replicates, 104 flies each					
Piperonyl butoxide plus *trans* fraction	40.00	400.0	43.65	100	92.6
	26.67	266.7	29.10	99.3	81.3
	17.78	177.8	19.40	94.3	47.0
	11.85	118.5	12.93	90.1	23.5
	7.901	79.01	8.621	83.4	6.7
"*cis*" fraction	60.00	600.0	67.45	98.8	90.5
	40.00	400.0	44.96	99.3	72.9
	26.67	266.7	29.98	95.3	36.7
	17.78	177.8	19.99	56.5	8.0
	11.85	118.5	13.32	28.6	3.2
"*cis*"-*trans* mixture	40.00	400.0	44.24	99.4	82.9
	26.67	266.7	29.50	98.1	53.0
	17.78	177.8	19.66	93.7	23.7
	11.85	118.5	13.11	72.4	8.4
	7.901	79.01	8.739	26.4	3.0
allethrin	39.51	395.1	43.70	98.3	89.6
	26.34	263.4	29.13	99.0	66.9
	17.56	175.6	19.42	96.9	30.7
	11.71	117.1	12.95	59.7	7.1
	7.804	78.04	8.631	40.0	1.0
Allethrin	200.0	—	200.0	100	91.9
	133.3	—	133.3	100	83.9
	88.89	—	88.89	100	63.3
	59.26	—	59.26	99.8	35.2
	39.51	—	39.51	99.0	14.4
trans Fraction	200.0	—	200.0	100	93.7
	100.0	—	100.0	100	77.8
	50.0	—	50.0	99.8	35.0
	25.0	—	25.0	98.4	7.9
Series 3. Seven replicates, 110 flies each					
Piperonyl butoxide	—	18,000	—	17.7	92.4
	—	12,000	—	5.4	55.6
	—	8,000	—	3.3	42.6
	—	5,333	—	4.7	24.9
	—	3,556	—	6.0	13.8
Allethrin	200.0	—	200.0	100	90.8
	133.3	—	133.3	100	77.1
	88.89	—	88.89	100	53.3
	59.26	—	59.26	100	27.7
	39.51	—	39.51	97.7	10.3
Pyrethrins	759.4	—	—	100	95.2
	506.3	—	—	100	81.0
	337.5	—	—	100	74.4
	225.0	—	—	100	51.5
	150.0	—	—	100	30.8
	100.0	—	—	100	22.3

[a] Allethrinoid equivalents of piperonyl butoxide are 0.01830 (*l-d* ester) 0.07681 (*d-l* ester), 0.4736 (*l-l* ester), 0.003148 (*d-d* ester), 0.009114 (*trans* fraction), 0.01241 ("*cis*"-fraction), and 0.01060 ("*cis*"-*trans* fraction and allethrin).

fraction was redetermined with the same populations of flies as was used with its mixtures, this estimate (1.163), despite the fact that its error is about double that of the former estimate (1.334), is entered in the table and was used in pertinent calculations. The two estimates are not significantly different. Since the new estimate for the relative toxicity of piperonyl butoxide (0.00978 as toxic as allethrin) was made in a separate series, the unweighted mean, 0.01060, of this and the old estimate (0.01141, Gersdorff *et al.* 1951) was used in the calculations. Each allethrinoid equivalent of piperonyl butoxide was then calculated by means of this mean allethrin equivalent of piperonyl butoxide and the toxicity of the allethrinoid relative to allethrin. These equivalents are given in table 1.

The ratio of these relative toxicities of the mixtures to those of the allethrinoids will, when significantly greater than unity, furnish estimates of the intensity of synergism. These ratios, together with their standard errors, are given in table 2. The estimates may also be obtained directly by calculating all LC-50's of the mixtures to allethrin equivalents as in table 2 and obtaining their inverse ratio to the LC-50 of the allethrin standard. Since the requirements to demonstrate synergism are properly made on the log concentration scale, also given in table 2 are the logarithms of the intensity ratios and the minimum logarithm required to demonstrate synergism. The evidence of synergism was pronounced in all mixtures.

Discussion of Synergistic Effects.—The piperonyl butoxide mixtures conform to the general pattern found in similar studies in that their regression lines are steeper than those for the allethrinoids alone, inasmuch as the latter have been found to be essentially parallel to that for allethrin (Gersdorff & Mitlin 1952, 1953). Therefore, the intensity of synergism will differ somewhat at different mortality levels. The ratios estimated from LC-5's and LC-95's determined from the equations based on equivalents are also given in table 2.

It is immediately apparent in table 2 that the intensity of synergism at the 50% mortality level is about threefold and not significantly different in the mixtures for all but one of the isomers as well as for the *trans* fraction. However, since this fraction is composed of these four isomers, the fourth isomer should also be synergized to the

Table 2.—**Evaluation of the relative effects on toxicity of piperonyl butoxide in mixtures with the *trans* isomers of allethrin.**

Material	LC-50 (Mg. per Dl.) Original Basis[a]	LC-50 (Mg. per Dl.) Allethrinoid Equivalent Basis	LC-50 (Mg. per Dl.) Allethrin Equivalent Basis	Relative Standard Error of Original LC-50 (Per Cent)	Relative Toxicity of Mixture on Allethrinoid-Equivalent Basis	Relative Toxicity of Principal Toxicant Alone[b]	Intensity of Synergism at 50% Mortality Level	Log of Intensity Ratio	Minimum Log Required to Demonstrate Synergism	Range of Intensity Ratio Between 5 and 95% Levels
				Series 1						
Piperonyl butoxide plus—										
l-d ester	32.88 + 328.8	38.90	22.53	4.18	1.933	0.5792	3.34±0.18	0.523	0.0456	2.2–5.0
d-l ester	103.3 +1033.0	182.6	25.21	4.35	0.4117	0.1380	2.98±0.15	.474	.0435	2.0–4.5
l-l ester	456.0 +4560.0	2616.0	58.55	4.42	0.0287	0.0224	1.28±0.068	.109	.0449	0.9–1.9[c]
d-d ester	7.052+ 70.52	7.274	24.49	4.04	10.335	3.367	3.07±0.17	.487	.0471	2.0–4.6
allethrin	22.41 + 224.1	24.79	24.79	4.36	3.033	1.0	3.03±0.17	.482	.0466	2.0–4.6
Allethrin	75.18	75.18	75.18	1.79	1.0	1.0	—	—	—	—
				Series 2						
Piperonyl butoxide plus—										
trans fraction	17.69 + 176.9	19.30	22.45	2.35	3.862	1.163	3.32±0.11	0.521	0.0274	2.5–4.3
"*cis*" fraction	31.46 + 314.6	35.38	30.20	2.40	2.107	0.854	2.47±0.08	.392	.0274	1.9–3.2
"*cis*"-*trans* mixture	24.91 + 249.1	27.55	27.55	2.43	2.705	1.0	2.71±0.09	.432	.0280	2.1–3.5
allethrin	22.20 + 222.0	24.55	24.55	2.39	3.036	1.0	3.04±0.10	.483	.0278	2.3–4.0
trans Fraction	64.07	64.07	74.53	4.35	—	1.163	—	—	—	—
Allethrin	74.53	74.53	74.53	3.08	1.0	1.0	—	—	—	—
				Series 3						
Piperonyl butoxide	8721.0	—	—	6.08	—	0.00978	—	—	—	—
Allethrin	85.27	—	—	6.23	—	1.0	—	—	—	—
Pyrethrins	210.5	—	—	4.93	—	0.405	—	—	—	—

[a] For the compound expressions the first figure refers to the allethrinoid.
[b] Although the *l-l* ester is not the principal toxicant in its mixture, the figure refers to the ester.
[c] Not significantly different from 1.0; synergism is not demonstrated until the ratio reaches 1.11, which it does at the 28% mortality level.

same degree. That the failure to achieve a sufficiently high estimate of the ratio with the *l-l* isomer mixture is an artifact of the procedure itself, and that an intensity approximately threefold is achieved with respect to this isomer when combined with the others to form the *trans* fraction, may be demonstrated from the data. This may be done with the use of the estimates for the relative toxicities of the mixtures and the principal toxicants, or more simply if it be remembered that the intensities of synergism are the expressions of the ratios of the toxicities of the mixtures to that of allethrin when the LC-50's are calculated on the allethrin-equivalent basis. These may be used in an equation based on the fact that the ratio of toxicity of the synergized *trans* fraction—that is, its allethrin equivalent—may be calculated as the sum of the allethrin equivalents of its components as determined from the ratios for the synergized isomers and their proper proportions. The proportions used are those found for the racemic pairs in a previous study (Gersdorff & Mitlin 1952). It was found by bioassay that 27% of the *trans* fraction consisted of the *alpha-dl-trans* isomer (the *l-d* and *d-l* pair) and 73% consisted of the other pair. The equation is as follows:

$$(0.135)(3.34)+(0.135)(2.98)+(0.365)(1.28)+(0.365)(3.07)=2.44$$

Such a ratio is obviously too low. Actually, it may be calculated from a similar equation that, to achieve a threefold ratio for the combined fraction, an intensity of 2.81 would be required for the *l-l* ester mixture, over twice that demonstrated in the tests. Why, then, does the estimate for this mixture fall appreciably short of this value? Since the slope of the regression line for the mixture is steeper than that for the isomer alone (which has been shown to be parallel with that for allethrin alone, Gersdorff & Mitlin 1953), this increase of effect with increase in concentration might at first be thought responsible and that we are faced with an example of a threshold of synergism. Moreover, it can be calculated from the equations and the error of the ratio that synergism is not demonstrated in this mixture below the 28% mortality level and that its intensity would be threefold theoretically at the 99.97% level. However, were the conditions of testing so changed as to bring the latter area of relationship within practical limits, the estimate of intensity would still not agree with those for the other isomers and the *trans* fraction, because the slopes of their regression lines are parallel to that for the *l-l* ester and the slopes of the lines for their mixtures are parallel to that for the *l-l* ester mixture.

The toxicity of the *l-l* ester being so low, piperonyl butoxide is responsible for about five-sixths of the toxicity of the mixture with that ester. Therefore, an inaccurate estimate of the relative toxicity of the synergist could seriously affect the estimate of the relative toxicity of the mixture. However, in series 3 the LC-50 of piperonyl butoxide is as precisely determined statistically as is that for allethrin, although it must be emphasized that such an estimate necessarily ignores the unmeasurable effect on strict comparability due to the larger particles in the piperonyl butoxide mist that result from the high concentrations required. The new estimate of the relative toxicity of the synergist, 0.00978, compares very favorably with the old estimate, 0.01141. The use of the individual equations for piperonyl butoxide and allethrin with regression coefficients of 3.170 and 3.721, respectively, would have little effect on the results, for the new estimates for the respective LC-50's would be 8766 and 85.35 and that for the relative toxicity of piperonyl butoxide 0.00974. Moreover, it may be calculated that to achieve a threefold intensity ratio, the LC-50 on the equivalent basis for the *l-l* ester mixture would have to be

1120 mg. per deciliter. For the LC-50 found on the original basis this would require an estimate of the toxicity of piperonyl butoxide relative to allethrin of 0.00326—that is, one-third that actually found. As will be deduced below, the effect of increased particle size is to lower the estimate of relative toxicity; therefore, were it possible to test piperonyl butoxide under strictly comparable conditions, its toxicity would be found higher, not lower, than that reported. Therefore, neither statistical error nor error due to increased particle size in the estimate for the relative toxicity of the synergist can be responsible for the low value for intensity obtained in this mixture.

When loss of comparability is considered with respect to the tests with the mixtures themselves, however, the difficulty is resolved. The toxicity of the *l-l* ester being so low, relatively high concentrations of its mixture with piperonyl butoxide are required for the tests. Many materials—and piperonyl butoxide is one of the outstanding ones according to R. A. Fulton of this laboratory—when incorporated in mixed sprays at high concentrations may greatly increase the size of the droplets of the spray mist. The range of concentrations in the *l-l* ester mixtures is five times that of the next lower range—that for the *d-l* ester mixtures—reaching about 14% in content of piperonyl butoxide. Such concentrations are high enough to result in much larger droplets in the spray mist than in the tests with the other mixtures. In accordance with Stokes' Law, that the falling time of the droplet is inversely proportional to the square of its diameter, the settling of the mist would be more rapid with the *l-l* ester mixture, and consequently a larger proportion of spray would be collected on the slide before its removal to begin the exposure period. Therefore, the amount received by the flies would be less than in the tests with the other materials. Thus strict comparability of the tests would be lost. Less spray would result in a lower dosage of insecticide. Consequently, a lower mortality than would have been caused under comparable conditions would be associated with the original concentration. Finally, the estimate for the relative toxicity of the mixture and, therefore, that for the intensity of synergism would be lower than they should be. It appears likely, then, that the difficulty in the demonstration stems from the low toxicity of the *l-l* ester.

In series 2 the intensity of synergism found for the "*cis*"-fraction is significantly lower than threefold. Whether this is due to the possible impurity of the sample of the "*cis*"-fraction used or is characteristic of the *cis* isomers must be determined by further work.

That the value found is not simply an experimentally low estimate is also demonstrated by combining the estimates of the two fractions according to their proportions as previously reported in this sample of allethrin (Gersdorff & Mitlin 1952), and comparing with that actually found by experiment with their mixture, as follows:

$$(0.31)(3.32)+(0.69)(2.47)=2.73;\ \text{found, } 2.71.$$

That the estimate for the *l-l* ester mixture is too low may also be demonstrated here in a comparison with the allethrin mixture through the use of the *trans*-isomer estimates, that for the "*cis*"-fraction in series 2 and the proportions of each, as follows:

$$0.31[(0.135)(3.34)+(0.135)(2.98)+(0.365)(1.28)+(0.365)(3.07)]+(0.69)(2.47)=2.46.$$

This intensity ratio is not only low as compared with that for the actual allethrin mixture (3.04) but also for the mixture containing the composite allethrin (2.71).

The results with allethrin and its mixture with piperonyl butoxide in the two series show that the two groups of fly populations were extremely similar in susceptibility.

EVALUATION OF RELATIVE KNOCKDOWN EFFECTIVENESS.—The final equations, showing the regression of percentage knockdown, expressed in probits, on concentration in milligrams per deciliter, expressed as logarithms, are given below. Since piperonyl butoxide alone caused only negligible knockdown, and in consequence would contribute little, if any, to the knockdown stimulus of the mixtures, except perhaps synergistically, no equivalents were calculated and the concentrations in the equations refer to those of the allethrinoidal materials only. The standard errors of the regression coefficients were 0.20 in series 1 and 0.36 in series 2.

Series 1:

Piperonyl butoxide plus—	
l-d ester	$Y=4.756X-0.060$
d-l ester	$Y=4.756X-4.093$
l-l ester	$Y=4.756X-9.433$
d-d ester	$Y=4.756X+3.684$
allethrin	$Y=4.756X+0.233$

Series 2:

Piperonyl butoxide plus—	
trans fraction	$Y=4.727X+1.334$
"*cis*" fraction	$Y=4.727X-0.572$
"*cis*"-*trans* mixture	$Y=4.727X+0.372$
allethrin	$Y=4.727X+0.448$

From these equations median effective concentrations (DC-50's) were calculated; they are given in table 3 together with other pertinent calculations.

DISCUSSION OF RELATIVE KNOCKDOWN EFFECTIVENESS.—The toxicity relative to the allethrin mixture with piperonyl butoxide was calculated for each of the other mixtures in table 2 and is also given in table 3 for comparison with the relative knockdown effectiveness. Despite the admitted crudeness of the knockdown criterion in such tests, there is no great difference between the two types of measurement. Initial paralysis and lethal action appear to be, then, not two separate, distinct types of toxic action, but phases of a single physiological reaction If ratios of toxicity based only on allethrinoid content are obtained, there is little choice between these and the relative toxicities as approximating knockdown effectiveness for four of the mixtures (those ratios approaching unity). However, where the divergence from unity is greater (for the mixtures of the *d-l*, *l-l*, and *d-d* esters), the ratios based on allethrinoid content differ more from the relative knockdown effectiveness. Moreover, with the least toxic mixture (that of the *l-l* ester) the ratio (0.049) differs greatly and is over twice as great as the toxicity of the isomer alone relative to allethrin alone, which is patently an incorrect expression. Therefore, despite the failure of piperonyl butoxide to contribute to knockdown (unless synergistically), the contribution of this material to toxicity must be considered in a comparison of relative toxicity and relative knock-

Table 3.—Evaluation of relative knockdown effectiveness of the mixtures of piperonyl butoxide with the *trans* isomers of allethrin and certain combinations of them.

MATERIAL	DC-50 (MG. OF ALLETHRINOID PER DL.)	RELATIVE KNOCKDOWN EFFECTIVENESS	RELATIVE TOXICITY[a]	RATIO OF RELATIVE KNOCKDOWN EFFECTIVENESS TO RELATIVE TOXICITY
		Series 1		
Piperonyl butoxide plus—				
l-d ester	11.58± 0.51	0.868 ±0.053	0.637	1.36
d-l ester	81.7 ± 3.0	0.123 ±0.007	0.136	0.90
l-l ester	1088.0 ±51.0	0.0092±0.0006	0.0095	0.97
d-d ester	1.89± 0.10	5.32 ±0.36	3.41	1.56
allethrin	10.05± 0.43	1.0	1.0	1.0
		Series 2		
Piperonyl butoxide plus—				
trans fraction	5.96± 0.49	1.540 ±0.156	1.272	1.21
"*cis*" fraction	15.10± 0.88	0.609 ±0.051	0.694	0.88
"*cis*"-*trans* mixture	9.53± 0.57	0.964 ±0.082	0.891	1.08
allethrin	9.19± 0.55	1.0	1.0	1.0

[a] Calculated from data in table 2 in comparison with the allethrin mixture.

down effectiveness. To gain precise information on the possibility of a synergistic effect on knockdown effectiveness by direct means, tests planned to study knockdown with the isomers alone or at least allethrin alone would be required. However, a conclusion regarding the effect may be reached from the data indirectly through two lines of reasoning and will be given later.

The toxicity relative to the allethrin mixture of each isomer mixture (that considering also the toxicity of piperonyl butoxide) is very close to the toxicity of that isomer relative to allethrin except in the case of the mixture of the *l-l* ester and then the ratio is lower because of, and to the same degree as, the lower intensity of synergism found in this case under the conditions of these tests.

If the LC-50 on the allethrinoid equivalent basis be divided by the DC-50 for each mixture with piperonyl butoxide in series 1 and 2, the following nine ratios are obtained: 2.9, 2.2, 2.4, 3.8, 2.5, 3.2, 2.3, 2.9, and 2.7, with a mean of 2.8. Thus, the intensity of the stimulus required to cause 50% mortality is about three times that required to cause 50% knockdown. Since the slopes of the regression lines for knockdown are very nearly the same as those for mortality, this generalization would hold true at other levels of effect.

If the mean ratio of LC-50 to DC-50 found for the mixtures be used to calculate conditional DC-50's from the LC-50's of allethrin and the *trans* fraction when used alone, the conditional DC-50's would be 26.9 and 26.6 mg. per deciliter for allethrin and 22.9 for the fraction (75.18, 74.53, and 64.07 divided by 2.8). These conditional concentrations, then, approximate those that would be required to demonstrate a synergistic action of threefold intensity with respect to knockdown. But from series 1 and 2, table 1, these concentrations cause knockdowns of about 96 and 98% and apparently the ratio of LC-50 to DC-50 for the toxicants alone is much greater than 2.8. In fact, if the difference between the ratio for the mixtures and that for the toxicants alone is due solely to the existence of synergism with respect to lethality but not to knockdown effectiveness, the ratio for the unsynergized toxicants would be 3.0 times 2.8, or 8.4. The above LC-50's for the toxicants alone when divided respectively by 10.05, 9.19, and 5.96, which are the DC-50's for the same toxicants in their mixtures and would be equal to the DC-50's for the toxicants alone if piperonyl butoxide were inert, furnish estimates of 7.5, 8.1, and 10.8, with a mean of 8.8. It may be concluded, then, that piperonyl butoxide exerts no synergistic action on knockdown effectiveness as measured in this way.

The DC-50's for the toxicants alone may be estimated approximately by inference. If DC-99's are determined from the equations for the mixtures with piperonyl butoxide and compared with DC-50's, it is found that the latter are 0.324 the former in series 1 and 0.322 in series 2. Since there is no significant difference between the slopes of the regression lines for toxicity and those for knockdown effectiveness for the mixtures, this relationship should be practically the same for the corresponding LC's. If the equations as given are used the ratios are 0.318 and 0.315 but if new equations were derived for toxicity with the regression coefficients the same as in the equations for knockdown effectiveness, the LC ratios would be identical with the DC ratios. The use of these equations has no effect on the estimate of relative toxicity, for the LC-50's as determined from them are almost identical with those reported in table 2. Since the proportion between the concentrations at the two levels is the same whether the latter are for knockdown or for mortality with the nine mixtures, a similar relationship probably exists with allethrin and the *trans* fraction alone, the proportion differing only because of the difference in the slopes of their lines. The LC-99's calculated from the equations, are for allethrin, 420.2 mg. per deciliter in series 1 and 345.7 in series 2, and for the *trans* fraction in series 2, 297.2. The ratio of the LC-50 to the LC-99 is then calculated as 0.179 in series 1 and as 0.216 in series 2. The concentrations causing 99% knockdown (39.5 mg. per deciliter for allethrin in both series 1 and 2, table 1, and 25.0 for the *trans* fraction) multiplied by these ratios thus furnish estimates of DC-50's for these

materials. They are, respectively, 7.07, 8.52, and 5.39 mg. per deciliter. When these values are compared with the pertinent values in table 3, the knockdown effectiveness of the allethrin mixture and that of the *trans* fraction relative to the toxicant alone are then calculated to be 0.70 and 0.93 for the former and 0.90 for the latter. Since the relative effectiveness of the mixtures must be significantly greater than unity to demonstrate synergism, the absence of synergism in the knockdown action of these mixtures is again indicated. Because piperonyl butoxide did not contribute to expected knockdown effectiveness, the DC-50's of the other mixtures expressed as allethrin equivalents are the same as that of the allethrin mixture and the conclusion is also applicable to them.

SUMMARY.—A comparative study was made of the toxicity, synergistic activity, and knockdown effectiveness of mixtures of piperonyl butoxide with allethrin and its *trans* fraction and isomers when applied to 10:1 proportion as space sprays on the Campbell turntable against house flies, *Musca domestica* L.

The mixtures with the *l*-allethrolone *d-trans* chrysanthemumic acid ester, the *d-l* ester, the *l-l* ester, the *d-d* ester, the *dl-trans* fraction, and allethrin were, respectively, 1.93, 0.41, 0.029, 10.34, 3.86, and 3.03 as toxic on the basis of equivalents as allethrin alone. The first five in the same order were 0.64, 0.14, 0.01, 3.41, and 1.27 as toxic as the allethrin mixture; except for the *l-l* ester, these are the same relationships as were found previously for the unsynergized toxicants.

Synergism with respect to mortality was demonstrated in all mixtures. The intensity of synergism was threefold at the 50% mortality level in each isomer mixture, in the *dl-trans* fraction mixture, and in the allethrin mixture. This intensity was indicated by deduction for the *l-l* isomer but could not be demonstrated experimentally, probably because of the relatively low toxicity of the isomer. Because of differences in slopes of regression lines, the intensity varied from two to nearly five between the 5 and 95% levels. In all mixtures there was no synergistic action with respect to knockdown in 25 minutes.

Relative knockdown effectiveness for the mixtures was approximately the same as their relative toxicity. The conclusion is made that initial paralysis and lethal action are not separate, distinct types of toxic action, but are phases of a single physiological reaction.

In the mixtures the intensity of the stimulus required to cause a given mortality was about three times that required to cause the same percentage knockdown in 25 minutes.

REFERENCES CITED

Bliss, C. I. 1939. The toxicity of poisons applied jointly. Ann. Appl. Biol. **26**: 585–615.

Finney, D. J. 1952. Probit analysis. 318 pp. Cambridge.

Gersdorff, W. A., and Norman Mitlin. 1952. A bioassay of some stereoisomeric constituents of allethrin. Jour. Washington Acad. Sci. **42**(10): 313–8.

Gersdorff, W. A., and Norman Mitlin. 1953. Effect of molecular configuration on relative toxicity to house flies as demonstrated with the four *trans* isomers of allethrin. Jour. Econ. Ent. **46**(6): 999–1003.

Gersdorff, W. A., R. H. Nelson, and Norman Mitlin. 1951. The relative effect of several pyrethrum synergists in fly sprays containing allethrin. Jour. Econ. Ent. **44**(6): 921–7.

LaForge, F. B., Nathan Green, and M. S. Schechter. 1954. Allethrin. Resolution of *dl*-allethrolone and synthesis of the four optical isomers of *trans*-allethrin. Jour. Organ. Chem. **19**(3): 457–62.

A Comparison of Injury Caused by the Apple Grain Aphid and Greenbug to Small Grains[1]

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The two most important aphids attacking small grains in the United States are the greenbug, *Toxoptera graminum* (Rond.), and the apple grain aphid, *Rhopalosiphum fitchii* (Sand.).

Several workers have reported that the greenbug caused greater damage to small grains in proportion to their number than other species of aphids. Wadley (1929) concluded that it was unlikely that the extraction of plant juices by greenbugs was greater than that by the apple grain aphid and suggested that a secretion of a chlorophyll-destroying enzyme may contribute to the damage caused by greenbugs. Chatters & Schlehuber (1951) studied the mechanics of greenbug feeding and the difference in injury to plant cells of barley, oats and wheat, and attempted to associate morphological plant characters with resistance or susceptibility. They found that greenbug damage varies from lysis in *Hordeum*, cell-wall modification in *Avena*, to a combination of lysis and cell-wall modification in *Triticum*, and concluded that injection of saliva and not the uptake of food appears to be the primary cause of tissue damage.

No detailed cytological study of the injury by apple grain aphids to small grains has been published, but since little or no chlorosis is caused by their feeding, it has usually been considered that injury by this aphid is due primarily to extraction of plant juices and that there is little if any injection of toxic substances.

To obtain a better understanding of the difference in injury caused by the apple grain aphid and greenbug a direct comparison of damage caused by these two species to three small grain crops was made under greenhouse conditions at Stillwater, Okla., in 1952 and 1953.

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MATERIALS AND METHODS.—Wintok oats, Pawnee wheat and Tenkow barley were seeded separately in 6-inch pots containing Reinach sandy loam soil fairly high in organic matter, with physical properties suitable for work in the greenhouse. After the seed was planted, the pots were placed in a metal pan containing 2 to 3 inches of water and left until the moisture had reached the soil surface. This method of planting and watering prevented movement of the seed prior to germination and excellent emergence was obtained within 4 to 6 days.

Four days after emergence the seedlings were thinned to five per pot. An effort was made to select uniformly vigorous seedlings with approximately equal space between each. Two weeks after seeding when the plants were approximately 5 inches tall, 5 pots of each crop were taken at random to use as uninfested checks, and 5 of each were infested with twenty-five 3- to 5-day-old apple grain aphid nymphs. After infestation, all plants in each pot, including the checks, were placed under cylindrical cages 12 inches long and 5 inches in diameter made of transparent cellulose nitrate plastic 0.02 inch thick. One end of the cage was closed with coarse mesh cloth, and the other end was placed in the soil around the plants. The height of each plant was recorded before infestation and at weekly intervals thereafter. The pots were arranged on the greenhouse bench as five replications in randomized block design. Greenhouse temperatures were regulated manually and usually ranged from 60° to 80°, with an average of 70.9° F.

To determine the tolerance to the two species of aphids, each plant was rated at 2-day intervals as follows according to the estimated percentage of leaf area damaged:

RATING	PER CENT DAMAGE
0	0–10
1	11–20
2	21–40
3	41–60
4	61–80
5	Beyond Recovery

All aphids on the plants within each randomized block were killed when the greenbug infested plants reached an average rating of 4. This was done by adding 50 cc. of a 0.017% aqueous emulsion of demeton to each pot. This same treatment was given the uninfested check plants. All of the pots, including the checks, were then exposed to a temperature of 19° F. for 4 hrs., and returned to the greenhouse for 16 weeks. The number of tillers on each plant was recorded at the end of 12 and 16 weeks.

In a fecundity test three plants of the same crop were grown in a 6-inch pot and each plant was caged separately. An alate aphid having just reached the adult stage was placed on each plant. Five such pots (15 plants) were used for each of the three crops, and tests were run concurrently but on separate plants with both greenbugs and apple grain aphids. These cages consisted of transparent cellulose nitrate plastic 0.01 inch thick made into tubes 6 inches long and 2½ inches in diameter. The number of nymphs produced by each alate female was determined at the end of 7 days.

After rates of reproduction data were obtained, one large cage as used in the tolerance test was placed over all the plants in each pot. Daily damage ratings were made on each plant until an average condition of 3 was reached. The aphids were then killed with demeton as previously described and the cages removed. Plant recovery was rated by the rapidity that new growth displaced injured tissue, and by the number of tillers per plant at 12 and 16 weeks after the aphids were removed.

RESULTS.—The results of comparison of injury and recovery of the three small grain crops when subjected to greenbug and apple grain aphid infestations are shown in table 1. Both species of aphids showed a very rapid population increase on all host plants and within 2 weeks after the plants were infested the aphids showed a tendency toward dispersal by concentrating on the sides of the cages.

Injury to the hosts by the apple grain aphid appeared to be a result of severe depletion of plant juices with a slight chlorotic effect rather uniformly over the entire plant. Greenbug damage in contrast started in localized spots surrounding the feeding punctures. Such spots merged as they became more numerous.

Pawnee wheat was injured very severely by greenbugs and within 16 days after being infested with 5 nymphs per plant, 79% of the tissue was chlorotic, and the plant heights averaged 3 inches less than the check. Although there was some stunting caused by apple grain aphids, only slight chlorosis was evident when the aphids were removed at the end of 16 days. Only 4% of the wheat plants injured by greenbugs recovered from the 4-hour exposure to 19° F., as compared with 64% of those that had been infested with apple grain aphid, and 84% of uninfested plants in the check. The rate of recovery as indicated by tillering was much slower for greenbug injured wheat plants than for those injured by apple grain aphid, but 16 weeks after the aphids were removed there were almost as many tillers on the plants that had been infested with greenbugs and apple grain aphids as on the uninfested check. Greenbugs and apple grain aphids reproduced at approximately the same rate on Pawnee wheat.

Tenkow barley was also injured much more severely by greenbugs than apple grain aphids and plants of this variety were stunted more than Pawnee wheat plants under similar conditions. Plants that had been infested with apple grain aphids survived the cold treatment slightly better than the uninfested plants and much better than those that had been infested with greenbugs. Rate of recovery as indicated by tillering was slightly faster for greenbug injured Tenkow barley plants than for Pawnee wheat plants. Greenbugs reproduced slightly faster on Tenkow barley than the apple grain aphid and this probably was responsible for some of the difference in injury.

Wintok oats was injured more by the apple grain aphid than Pawnee wheat or Tenkow barley. The oats, however, were injured more severely by greenbugs than by the apple grain aphid. Wintok oats were resistant to freeze injury, and all plants in the check pots recovered. All but 4% of those that had been infested with the apple grain aphid recovered, however, 56% of the greenbug injured plants were killed. Greenbugs reproduced slightly faster on Wintok oat than on Tenkow barley and considerably faster than on Pawnee wheat.

Table 1.—Reaction of three small grain crops to greenbug and apple grain aphid infestations.

Insect	Five Nymphs per Plant					One Adult per Plant			
	Plant Condition When Aphids Were Removed		Plants Killed by 4 Hrs. Exposure to 19° F.	Average Tillers per Plant After Recovery		Progeny per Female in 7 Days	Time Required for Injury Rating of 3	Plant Recovery After Injury Rating of 3	
	Chlorotic Tissue	Height		12 Weeks	16 Weeks				
	Per Cent	Inches	Per Cent	Number	Number	Number	Days	Per Cent	Days
				Pawnee wheat					
Greenbug	79	9.2	96	3.7	7.8	12.9	24	87	24
Apple grain aphid	3	10.8	36	6.4	7.9	12.6	38	80	18
Check	0	12.4	16	6.9	8.1	—	—	—	—
				Tenkow Barley					
Greenbug	77	7.9	76	4.7	11.0	14.9	23	83	33
Apple grain aphid	5	10.7	32	6.7	11.2	11.3	37	87	17
Check	0	13.6	40	6.4	12.3	—	—	—	—
				Wintok Oat					
Greenbug	75	6.2	56	8.0	14.0	15.9	24	93	24
Apple grain aphid	10	7.0	4	10.0	14.5	11.9	37	57	19
Check	0	8.9	0	14.0	17.2	—	—	—	—

Sixteen weeks after the aphids were removed from Wintok oats there was an average of three tillers less per plant on those that had been infested by greenbugs and by apple grain aphids than on those plants that had been uninfested, indicating that permanent injury may have occurred.

SUMMARY.—In greenhouse studies damage caused to Pawnee wheat, Tenkow barley and Wintok oat by the apple grain aphid *Rhopalosiphum fitchii* (Sand.), was compared with damage caused by the greenbug, *Toxoptera graminum* (Rond.). The susceptibility to freeze injury of plants of the three cereals that had been infested with the two species of aphids was compared. Recovery after injury was measured by tiller development. Fecundity of the two aphid species on the three cereal varieties was compared.

Injury symptoms appeared on all three hosts infested with greenbugs much earlier than when infested with the apple grain aphid. Prolonged infestations by heavy populations of apple grain aphids eventually caused damage comparable in severity to greenbug injury. Plants that had been infested with greenbugs were much more susceptible to freeze damage than uninfested plants, and Pawnee wheat and Wintok oat plants that had been infested with apple grain aphids were slightly more susceptible to this type of damage than check plants. However, fewer Tenkow barley plants that had been infested with the apple grain aphid were killed by low temperatures than the uninfested plants.

Greenbugs feeding on plants caused a marked delay in formation of tillers. This delay appeared to be transitory in Pawnee wheat and Tenkow barley and was of a more permanent effect in Wintok oats.

Greenbugs reproduced faster on the three crops than the apple grain aphid but this difference on Pawnee wheat was very slight.

REFERENCES CITED

Chatters, R. M., and A. M. Schlehuber. 1951. Mechanics of Feeding of the Greenbug (*Toxoptera graminum* (Rond.) on *Hordeum*, *Avena*, and *Triticum*. Oklahoma Agric. Expt. Sta. Tech. Bull. T-40, 18 pp.

Wadley, F. M. 1929. Observations on the injury caused by *Toxoptera graminum* (Rond.) (Homoptera: Aphididae). Proc. Ent. Soc. Wash. **31**(7): 130–4.

Properties and Metabolism in the Cockroach and Mouse of Malathion and Malaoxon[1]

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Malathion (*O,O*-dimethyl *S*-(1,2-bis-carboethoxy) ethyl phosphorodithioate) is an insecticide of great interest because of its low mammalian toxicity. Furthermore, resistance to it by insects is not developed to the extent familiar with chlorinated hydrocarbons. The thiolphosphate analogue of malathion (*O,O*-dimethyl *S*-(1,2-bis-carboethoxy) ethyl phosphorothiolate) has been called "malaoxon" (O'Brien, 1956a) just as the corresponding analogue of parathion is called paraoxon. Malaoxon is of interest as a probable metabolic product of malathion in both insects and mammals, and its production may account for the toxic effects of malathion, in the same way that paraoxon accounts for the *in vivo* effects of parathion. Furthermore, malaoxon has been proposed as an insecticide and acaricide (Johnson 1953), as it lacks the odor of malathion. The present paper gives data on the properties and metabolism of malathion and malaoxon, and uses the information to propose a mechanism for the selective toxicity of malathion.

Materials and Methods.—Malathion was supplied as 99% pure compound by the American Cyanamid Company, New York. Malaoxon (S calculated 10.20, found 9.87; $N_D^{24} = 1.4648$) was prepared from it by the method of Johnson (1953).

Partition coefficients were found by equilibrating 5 ml. of appropriate solvent pairs containing the compounds, and determining the phosphorus in each phase by the method of Rockstein & Herron (1951), after digestion by a micromodification of the method of Simmons & Robertson (1950). In the determination, it was necessary to allow the samples to stand for 30 min. after adding the acid ammonium molybdate, in order to develop the full color. Since sodium phosphate developed color promptly, the organophosphate must be incompletely hydrolysed during digestion.

Hydrolysis rates were studied with an automatic titrator (Radiometer Model TTT2). Ultraviolet spectra were measured on ethanolic solutions with a Beckman DK spectrophotometer; infra-red spectra on smears using a Perkin-Elmer Model 21 spectrophotometer with rock-salt prism.

Anticholinesterase determinations were made by a standard Warburg procedure at 25° C. with $NaHCO_3$ at 3×10^{-2} M. The inhibitor was incubated with the enzyme for 90 minutes before adding substrate (acetylcholine bromide 1.47×10^{-3} M). For serum cholinesterase 1 ml. of 2.5×diluted human plasma was used; for fly cholinesterase, 1 ml. of a homogenate of 2.8 house fly heads per ml. of 0.9% KCl; for brain, 1 ml. of a homogenate of 1 mouse brain in 18 ml. of 0.9% KCl.

The insects used in these studies were the adult cockroach, *Periplaneta americana* (L.) or the adult house fly, *Musca domestica* L.

Results.—*Physical Properties.*—The partition coefficients of malathion and malaoxon are given in table 1. Clearly the oxidation greatly increases the partitioning into polar solvents. The same conclusion was reached from the results of submitting the compounds to paper chromatography using the silicone system developed for demeton by March *et al.* (1954). Each gave single spots, malathion having an R_F of 0.08 ± 0.012 and malaoxon an R_F of 0.890 ± 0.011. As the method employs a reverse phase system with a polar solvent, these results show malaoxon to be a far more polar compound than malathion.

The hydrolyses of the compounds were examined at various pH's. Malathion (in 50% ethanol) was not hydrolysed at pH's of 4, 6 or 8. At pH 10 slow hydrolysis was observed. Malaoxon was not hydrolysed at pH 4 or 6. At pH 8 a slow hydrolysis was observed; at pH 10 vigorous hydrolysis was found. The hydrolysis does not appear to be of a first-order form in either compound; the behaviour is qualitatively like that of diethyl malate, so that the carboxyester groups are probably those involved.

Table 1.—Partition coefficients of malathion and malaoxon

System	Malathion	Malaoxon
Chloroform-water	64	5.8
Hexane-water	19	0.42
Petroleum ether (B.P. 220–230° F.) –ethanol 95%	11	1.8
Carbon tetrachloride–water	39	2.9

The stability of malaoxon under strongly acid conditions was studied. Samples were added to 1% and 10% perchloric acid; after 30 minutes the perchlorate was precipitated with potassium carbonate, the mixture was filtered, and the pH adjusted to about 7.5. Aliquots were taken and assayed for anticholinesterase activity, and compared with malaoxon which had not been exposed to the acid. The treatment caused no loss in activity, and malaoxon was therefore stable under those conditions. Perchlorate precipitation of protein can thus be employed in extraction of malaoxon from tissues.

There was a possibility that malathion might be oxidized to malaoxon by perchloric acid. Malathion samples were added to 10% perchloric acid at 25° C. and 50° C., extracted after 1 hour with chloroform, and chromatographed. Malathion was the only compound found.

The spectra of malaoxon in the ultra-violet and infra-red regions were examined and compared with those for malathion, which have been described earlier (O'Brien 1956a). The solvent was absolute ethanol. There was a marked difference in the ultra-violet spectra as shown in figure 1, malaoxon having a broad shoulder with a plateau between 248 and 268 mμ. Figure 2 shows that the

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FIG. 1.—Ultraviolet spectra of malathion (A) and malaoxon (B).

infra-red spectrum of malaoxon differed from that of malathion in lacking a 655 cm.$^{-1}$ peak, having a reduced 820 cm.$^{-1}$ peak, and having additional peaks at 1625, 1570, 855, 775 and 795 cm.$^{-1}$. The absence of P→S accounts for the loss of the 655 peak (Daasch & Smith 1951) and possibly for the 820 peak (Henglein *et al.* 1954). In the 1200–1350 region where P→O vibration is usually seen (Williams 1951, Holmstedt & Larsson 1951) malaoxon showed no clear absorption peak which malathion lacked. However, both compounds absorbed strongly in this region, with a very blunt peak at 1265 for malaoxon.

Cholinesterase inhibition.—Table 2 shows the effectiveness of the two compounds against various cholinesterases *in vitro*. In all cases malaoxon was the more potent inhibitor.

Malaoxon as a product of malathion metabolism.—Metcalf & March (1953) have shown that roach gut converts malathion to a stronger cholinesterase *in vitro*. March *et al.* (1956) showed that in the intact mouse and hen malathion is converted to a number of chromatographically distinguishable products, whereas metabolism in the intact cockroach was far less extensive. They also showed that of the probable products which they synthesized, only malaoxon was a better anticholinesterase than malathion and of comparable toxicity to insects. However, their chromatographic technique, which was designed to separate out the ionized degradation products, would not distinguish malathion from malaoxon, and the latter was therefore not demonstrated as a product. It was therefore decided to investigate the possible production of malaoxon from malathion by mouse liver and cockroach gut.

Table 2.—Cholinesterase inhibition by malathion and malaoxon.

	MALATHION[a]	MALAOXON
I_{50}[b] against		
serum ChE	9.8×10^{-3}	4.5×10^{-7}
house fly head ChE	2.0×10^{-5}	4.6×10^{-9}
cockroach cord ChE	2.0×10^{-5}	1.2×10^{-8}
mouse brain ChE	4.2×10^{-5}	4.0×10^{-8}
Bimolecular rate constant[c]		
against serum ChE	—[a]	1.26×10^{4}

[a] Malathion does not inhibit in accordance with first-order kinetics (O'Brien 1956a).
[b] I_{50}: molar concentration for 50% inhibition of cholinesterase (ChE).
[c] Bimolecular rate constant calculated as in Aldridge (1950); units: litres moles^{-1} min.$^{-1}$

Attempts were made to identify malaoxon (a) in the solution remaining after incubating 25 cockroach guts or 2 sliced mouse livers with malathion (5×10^{-4} M) in aerated phosphate buffer for periods up to 3 hours (b) in homogenates (prepared in the Waring blendor) of whole mice injected with from 300 to 600γ/g., or of groups of 50 cockroaches injected with 100γ/g. of malathion. The mice were homogenized at death or at 5 to 15 minutes after injection; the cockroaches after 2 to 3 hours. In all cases, protein was removed by precipitation with per-

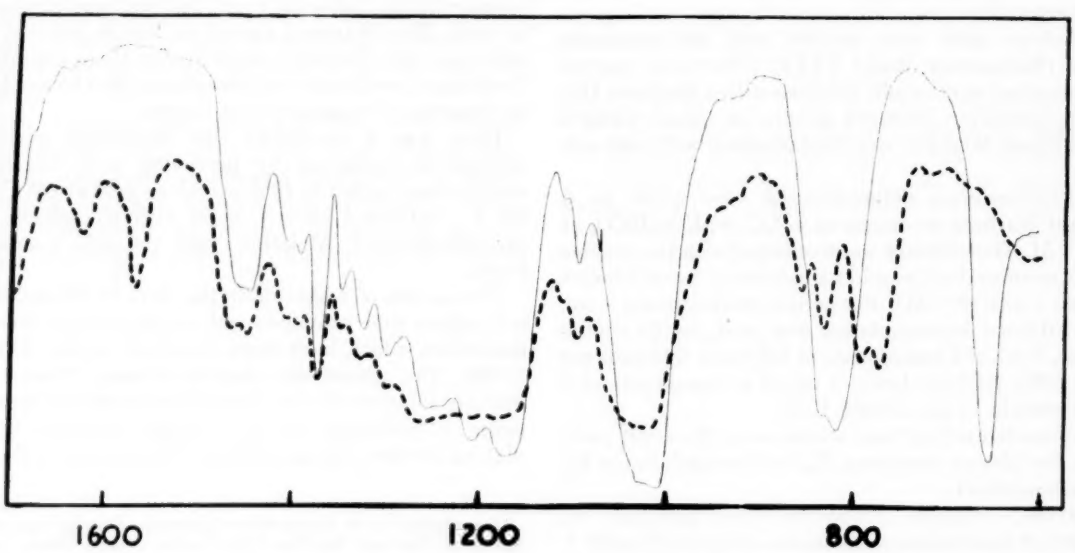

FIG. 2.—Part of the infra-red spectra of malathion (solid line) and malaoxon (broken line), using liquid smears. Ordinate: transmittance. Abscissa: wave number in cm^{-1}.

chloric acid, the solution was neutralized with KOH and extracted twice with chloroform, then acidified and extracted twice again. The chloroform extracts were reduced to 1 ml. and chromatographed with the demeton procedure of March *et al.* (1954).

In no case could malaoxon be detected, although malathion and ionized derivatives were always found (the ionized derivatives were those extracted from acid but not neutral solution. Malaoxon, if present, should be extractable from the neutral solution as is the case for malathion). The chromatographic procedure is relatively insensitive; the minimum detectable concentration of malaoxon was 10γ. The detection technique was the molybdate spray method of March *et al.* (1954).

Attempts were therefore made to demonstrate the presence of malaoxon by using methods based upon its inhibition of cholinesterase. As malathion is 20,000 times less effective than malaoxon as an inhibitor of serum cholinesterase (table 2), this enzyme was used. Extracts as in (a) above were studied. By observing the effect of time of incubation with ChE upon consequent inhibition, the bimolecular rate constants for inhibition were found (Aldridge 1950). The results were: cockroach gut product, 2.0×10^4 litres moles^{-1} min.$^{-1}$; mouse liver product, 5.4×10^4; malaoxon 1.3×10^4. Malathion does not inhibit in accordance with first-order kinetics (O'Brien 1956a). These results suggest that malaoxon was in the products.

Another approach was to compare the effectiveness of the inhibitor against different cholinesterases. The ratio of the potencies against the cholinesterases of human serum, mouse brain and fly head were: liver product, 1:5:68; cockroach gut product, 1:17:56. These compare with malaoxon 1:11:98, and malathion 1:233:490. Clearly the inhibitor involved was not malathion, but the agreement with the malaoxon pattern is only approximate.

Finally, the ultra-violet spectra of an ethanol solution of the metabolites was examined, and showed a broad shoulder in the 260 mμ region. This is in conformity with the hypothesis that the metabolite is malaoxon, although it is weak evidence in that numerous other potential metabolites may absorb in this region.

The results given above should be considered along with the observations that (1) both gut and liver produce a strong anticholinesterase from malathion (see below), and (2) of all the expected metabolites of malathion which have been synthesized, only malaoxon is a more potent anticholinesterase than the present product (March *et al.* 1956). The balance of evidence is thus in favour of the idea that malaoxon is a product of malathion metabolism in the mouse and roach. The final evidence may come from chromatography of radioactive metabolites. In the following discussion it will be assumed that malaoxon is the potent anticholinesterase derived from malathion oxidation.

Malaoxon production by tissue preparations.—Davison (1955) has shown that parathion can be oxidised by liver homogenates in the presence of diphosphopyridine nucleotide (DPN), nicotinamide and Mg^{++}, and that this activity resides in the combined microsomal and soluble fractions. These findings have been confirmed for malathion (table 3). The experiments tabulated here were studied by a method which has proved useful in studies on schradan oxidation (O'Brien & Spencer 1953). The tissue preparation is incubated in Warburg flasks exposed to the air along with necessary cofactors and serum cholinesterase. After 60 min., bicarbonate is added to the flasks, and they are placed on the manometers and gassed with 5% CO_2 in N_2. At 90 min., acetylcholine is tipped in and the cholinesterase activity measured. If malathion is degraded by the preparation, a cholinesterase inhibition smaller than that for malathion alone would be observed; if malathion is oxidised to malaoxon, a cholinesterase inhibition greater than that for malathion alone would be found.

Table 3.—Activation and degradation of malathion by mouse liver homogenates.[a]

Additions	Per Cent Cholinesterase Inhibition	Increased Per Cent Inhibition Due to Additions
(a) Nil	38	—
(b) Liver (1% frozen) with additions shown:		
nil	27	−11
cofactors	57	19
cofactors+extra DPN, 1 mg.	55	17
cofactors+DPNH, 1 mg.	63	25
cofactors+catalase, 1 mg.	61	23
(c) Microsomes (from 10% fresh liver) with additions shown:		
supernatant	25	−13
cofactors+supernatant	78	40
cofactors	67	29
cofactors+extra DPN (1 mg.)	67	29
cofactors+DPNH (1 mg.)	88	50
cofactors+DPNH (1 mg.) (anaerobic)	48	10

[a] Conditions: All have 1 ml. diluted plasma (cholinesterase source), malathion 5.0×10^{-4} M. Cofactors: Mg^{++} 0.018 M, nicotinamide 0.01 M, DPN 4.9×10^{-4} M. Catalase: General Biochemicals Inc. Microsomes washed once.

Davison (1955) showed that schradan oxidation by liver homogenates occurred under the same condition as for parathion oxidation. O'Brien (1956b) showed that in schradan oxidation by liver the soluble fraction served only to convert the DPN to the reduced form (DPNH), and that in the absence of soluble fraction, microsomes with DPNH could accomplish the oxidation.

Table 3 part "b" shows that the liver homogenate degraded malathion, but that on addition of cofactors (Mg^{++}, DPN, nicotinamide), an activation of malathion was observed, presumably due to oxidation to malaoxon. If DPNH was now added, a still greater activation was found, which was not due to raising of the DPN concentration following oxidation of the DPNH, since equivalent extra DPN was ineffective. The enhancement of activity by added catalase, reported for the schradan activation system by O'Brien (1956b) was observed for malathion activation, but to a smaller extent.

Table 3 part "c" shows that in the presence of cofactors even washed microsomes gave good activation of malathion. Addition of the supernatant fraction or of DPNH enhanced the activation. Again the DPNH effect was not due to its content of potential DPN. When cofactors were excluded, it was noted that a system responsible for degrading malathion was present in the combined soluble

Table 4.—Activation and degradation of malathion by cockroach guts, whole and homogenized.[a]

Additions	Per Cent Cholinesterase Inhibition	Increased Per Cent Inhibition Due to Additions
Nil	38	—
Whole gut	64	26
Whole gut (anaerobic incubation)	15	−23
Whole gut + phosphate buffer	67[b]	29
Gut homogenate	19	−19
+ cofactors	45	7
+ cofactors + DPNH	48	10
+ cofactors + catalase, 1 mg.	47	9

[a] Conditions as in table 3.
[b] Corrected for retention effect of phosphate buffer by comparing to a phosphate-containing system.

and microsomal fractions. Under anaerobic conditions, activation was greatly depressed.

Whole cockroach guts give excellent malathion activation in the Warburg system described above, as shown in table 4. Under anaerobic conditions, the activation was suppressed and a degradative activity revealed. Homogenizing the gut also eliminated malaoxon production and gave the degradation of malathion as was found for liver. The addition of Mg^{++}, nicotinamide and DPN restored the oxidizing activity to some extent, but extra DPNH showed only a very small additional production, in contrast to the findings for liver. This activation of gut homogenate by these cofactors is not observed in schradan oxidation (O'Brien 1957b). Furthermore, phosphate buffer had no effect upon malathion oxidation by whole guts, although it inhibits schradan oxidation by whole guts.

These data show that the malathion oxidation system of liver is very similar to the parathion and schradan oxidation systems in that tissue; it is in the microsomes, and requires DPNH or a combination of DPN and a reducing system, as well as Mg^{++} and nicotinamide. The malathion oxidation system of cockroach gut is to some extent like that of liver, in contrast to the schradan oxidising system which has different properties in cockroach gut and in liver.

The distribution of activating and degrading activity in various mouse and cockroach tissues.—The experiments reported above showed that in mouse liver and cockroach gut, systems were present which could degrade malathion to a less potent or activate it to a more potent anticholinesterase. Further work showed that malaoxon could also be degraded and is easier to study since it cannot be further activated.

Several tissues from the mouse and the cockroach were examined for malathion activating activity by adding malathion and cofactors (Mg^{++}, nicotinamide, DPN) to homogenates, and comparing the anticholinesterase activity with that given by malathion alone. Since the observed result is a balance of the processes of degradation and activation, the degradative capacity of the tissues was also examined, by adding malaoxon to homogenates. Thus where a high degrading activity was found, the failure to activate malathion does not necessarily indicate a low level of activating system.

The results (table 5) show that in the mammal the principal degrading activity is in liver, kidney and lung. The principal malathion activating activity is in liver, with some in heart and perhaps testis. This latter pattern is like that of schradan activation (O'Brien 1956b). In the insect only the fat body degrades malaoxon extensively; midgut and fat body are strong activators of malathion.

Cellular distribution and specificity of the degrading system.—Centrifugal studies on mouse liver homogenates showed that washed mitochondria or microsomes had no malaoxon degrading actvity; the whole activity resided in the supernatant after centrifuging at 45,000×g for 30 min.

As kidney was the most potent source of the degrading system, supernatant from a 10% homogenate was prepared as above (frozen kidney from the cat was used). To 2 ml. of this homogenate was added 0.3 ml. of 2.52% $NaHCO_3$. After gassing (5% CO_2 in N_2) malathion or malaoxon at 1.08×10^{-3} M was tipped in. For malathion, 20 µl of CO_2 per hour was released; for malaoxon, 4 µl per hour. At this concentration malathion is thus the preferred substrate for the system.

The balance of activating and degrading processes.—The results above have shown that there are in the mouse and the cockroach systems capable of activating and degrading malathion. It seemed possible that the selective toxicity of malathion might be due to different relative rates of the two processes.

Experiments were carried out with (a) one sliced mouse liver, (b) 25 whole cockroach guts, (c) 6 whole cockroaches with dorsal integument removed. In each case, the tissues were placed in 50 ml. of phosphate buffer (0.067M, pH 7.4) and aerated at room temperature. Seven mg. of malathion or 0.2 mg. of malaoxon in 0.5 ml. of ethanol were added to the buffer. Aliquots were removed at timed intervals and their anticholinesterase activity determined against serum cholinesterase. The results are given in figure 3.

The curves for malathion represent the balance of the two opposing processes of activation and degradation. The curves for malaoxon represent only the degradative process. The results show that in the cockroach degrada-

Table 5.—Activation of malathion and degradation of malaoxon by various tissues.[a]

Tissue Added	Per Cent Cholinesterase Inhibition by Malaoxon	Malaoxon Degrading Activity	Per Cent Cholinesterase Inhibition by Malathion	Malathion Activating Activity
(a) Nil	77		38	
(b) Mammal:				
liver	5	++++	49	(+++)
kidney	5	++++	16	?
brain	71	+	41	–
lung	10	++++	34	?
heart	77	–	65	++
testis	54	+	43	(+)
pancreas	70	+	39	–
spleen (rabbit)	66	+	39	–
(c) Insect:				
foregut	76	–	48	+
midgut	73	–	71	+++
hindgut	73	–	53	++
fat body	48	++	77	+++

[a] Conditions (except as shown). All have 1 ml. diluted plasma (cholinesterase source). Mammal: 0.5 ml. of 10% homogenates of frozen tissues from male cat. Insect: whole tissues from one cockroach. Malathion at 5.1×10^{-4} M, Malaoxon at 10^{-6} M. Cofactors, as in table 3, added with malathion.

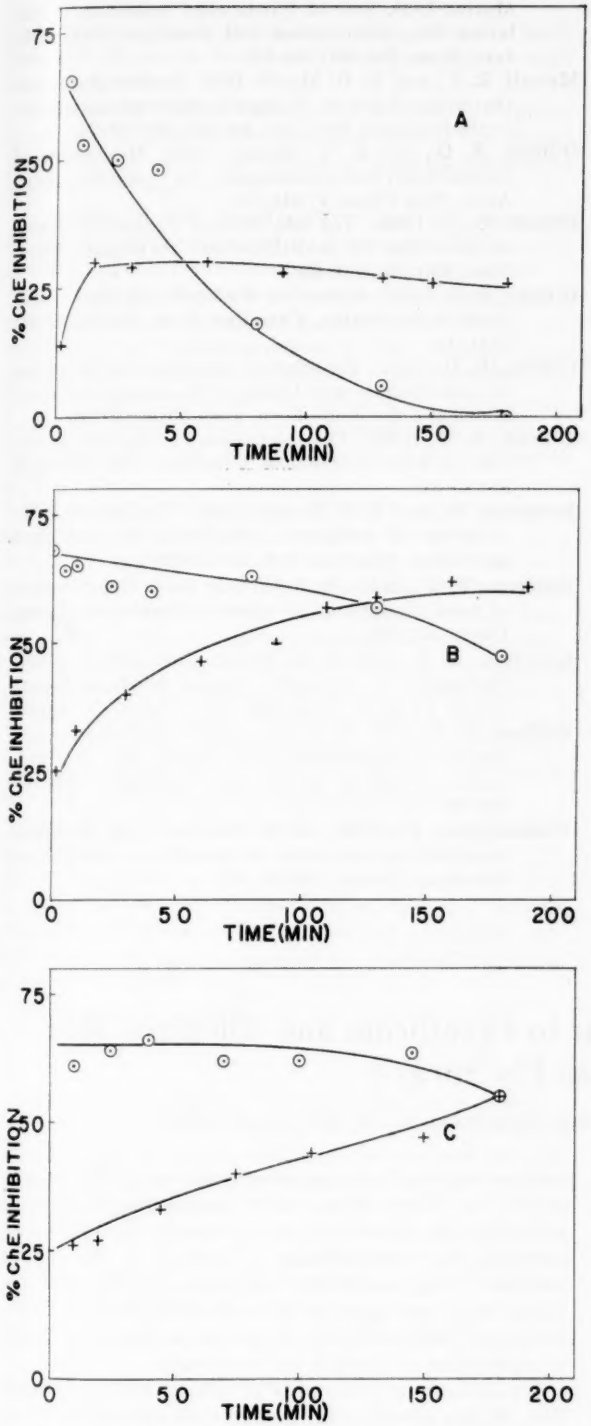

Fig. 3.—Anticholinesterase activity in buffered solutions of malathion (+) and malaoxon (⊙) incubated with: A. Mouse liver slices. B. Cockroach guts. C. Cockroaches with dorsum removed.

tion is very slight, and (perhaps as a result) a steady increase in overall anticholinesterase activity is observed. Presumably in the poisoned cockroach, malaoxon accumulates steadily. In mouse liver, the initial rapid activation is opposed by a vigorous degradation and after 15 minutes the balance of the two processes shows a slow reversal of the small initial rise in anticholinesterase activity. As a corollary, added malaoxon is very rapidly degraded, the process being complete in 3 hours. The data cannot be interpreted quantitatively because malaoxon degradation only has been studied, and malathion can be degraded directly, as shown in several experiments above.

As shown above, the liver is the most important organ for malathion activation, by mammals but other tissues (especially kidney) have a strong degradative capacity. Therefore, it is to be expected that in the intact mouse, degradation would be much more extensive, but activation would be little more than that observed for liver slices (even though slicing the liver probably damages the esterase activity less than the oxidative capacity).

These findings are in agreement with the chromatographic observations of March *et al.* (1956), who observed that in the mouse numerous degradation products of malathion were found. In the cockroach, there was less extensive degradation.

Discussion.—In a previous paper (O'Brien 1946a) it was suggested that the toxicity of malathion to insects might not be due to an anticholinesterase action. An important piece of evidence was that death in the poisoned cockroach or house fly occurred when the cholinesterase had recovered from the initial marked inhibition. However, Smallman & Fisher (1956) have now shown an identical pattern of cholinesterase inhibition and recovery in flies poisoned with a lethal dose of tetraethyl pyrophosphate; furthermore the acetyl choline level showed an initial sharp rise and then decreased as the cholinesterase level recovered, as has also been shown by Winteringham & Harrison (1956) with flies poisoned by a lethal dose of di-*iso*propyl fluorophosphate. Malathion, therefore, behaves as a typical organophosphate anticholinesterase. It is not a potent inhibitor of the enzymes of carbohydrate metabolism (O'Brien 1957a). If the assumption is made that the toxic effect of malathion and its metabolites is due to cholinesterase inhibition, the studies reported herein seem to explain satisfactorily the selective toxicity of the insecticide.

The balance studies on tissues *in vitro*, combined with the *in vivo* observations of March *et al.* (1956), strongly suggest that the non-toxicity of malathion to the mouse is due to the vigorous hydrolytic degradation of either malathion or malaoxon at the carboxylic ester link. This hydrolysis outstrips the process of malaoxon accumulation caused by oxidation of malathion. In the cockroach, activation is much more rapid than hydrolysis, and malaoxon presumably accumulates steadily to a lethal level.

Summary.—The stability, polarity, spectra and anticholinesterase activities of malathion and malaoxon are described.

Evidence is given that malaoxon is a product of malathion metabolism in the cockroach and mouse.

Mouse liver is shown to activate malathion, probably by oxidation to malaoxon, by means of a microsomal system requiring DPNH, Mg^{++} and nicotinamide. Although whole cockroach guts activate malathion, this property is lost on homogenization and only a little restored by addition of the above cofactors.

In the mammal, malathion oxidation occurs primarily in the liver, and to a lesser extent in heart and perhaps

testis. Malaoxon hydrolysis is vigorous in liver, kidney and lung.

In the cockroach, fat body and midgut oxidise malathion; fat body hydrolyses malaoxon.

The hydrolysing system of liver is in the soluble fraction, and is more active against malathion than malaoxon.

The balance of oxidative (activating) and hydrolytic (degrading) activities in the mouse and cockroach accounts satisfactorily for the much greater toxicity of malathion for the cockroach than for the mouse.

REFERENCES CITED

Aldridge, W. N. 1950. Some properties of specific cholinesterase with particular reference to the mechanisms of inhibition by E605 and analogues. Biochem. Jour. **46**: 451–60.

Daasch, L. W., and D. C. Smith, 1951. Infra-red spectra of phosphorus compounds. Analyt. Chem. **23**: 854–68.

Davison, A. N. 1955. The conversion of schradan and parathion into inhibitors of cholinesterase by mammalian liver. Biochem. Jour. **61**: 203–9.

Henglein, A., G. Schrader, and R. Muhlmann. 1954. Quantitative infrarotspektroskopische Bestimmung des Isomerenverhaltnisses in dem System-Insecticid "Systox." Zeitschr. Analyt. Chem. **141**: 276–81.

Holmstedt, B., and L. Larsson. 1951. Infra-red spectra of some organic phosphoryl compounds. Acta Chem. Scand. **5**: 1179–86.

Johnson, G. A. 1953. O,O-dialkyl S-(1,2-dicarbalkoxyethyl) monothiophosphates and miticide compositions containing the same. U. S. Patent 2,713,018.

March, R. B., R. L. Metcalf, and T. R. Fukuto. 1954. Paper chromatography of the systemic insecticides, demeton and schradan. Jour. Agric. Food Chem. **2**: 732–5.

March, R. B., T. R. Fukuto, R. L. Metcalf, and M. G. Maxon. 1956. Fate of P32-labelled malathion in the laying hen, white mouse and American cockroach. Jour. Econ. Ent. **49**: 185–95.

Metcalf, R. L., and R. B. March. 1953. Further studies on the mode of action of organic thionophosphate insecticides. Anns. Ent. Soc. America **46**: 63–74.

O'Brien, R. D., and E. Y. Spencer. 1953. Metabolism of octamethylpyrophosphoramide by insects. Jour. Agric. Food Chem. **1**: 946–50.

O'Brien, R. D. 1956a. The inhibition of cholinesterase and succinoxidase by malathion and its isomer. Jour. Econ. Ent. **49**: 484–90.

O'Brien, R. D. 1956b. Activation of schradan by mammalian tissue homogenates. Canadian Jour. Biochem. **34**: 1131–41.

O'Brien, R. D. 1957a. The effect of malathion and its isomer on carbohydrate metabolism of the mouse, cockroach and house fly. Jour. Econ. Ent. **50**(1): 79–84.

O'Brien, R. D. 1957b. The mechanism of schradan activation by liver microsomes. Canadian Jour. Biochem. **35**: 45–54.

Rockstein, M., and P. W. Herron. 1951. Colorimetric determination of inorganic phosphorous in microgram quantities. Analyt. Chem. **23**: 1500–1

Simmons, W. R., and J. H. Robertson. 1950. Determination of total phosphorus in organic phosphates. Analyt. Chem. **22**: 294.

Smallman, B. N., and R. W. Fisher. 1956. Effect of anticholinesterases on acetyl choline levels in insects. Tenth Internat. Congress Ent. Montreal, August.

Williams, E. F. 1951. Properties of *O,O,*-diethyl *O-p*-nitrophenyl thiophosphate and *O,O*-diethyl *O-p*-nitrophenyl phosphate. Indust. and Engineer. Chem. **43**: 950–4.

Winteringham, F. P. W., and A. Harrison. 1956. Studies of anticholinesterase action in insects by a labelled pool technique. Nature **178**: 81–83.

Comparative Effects of Piperettine in Pyrethrum and Allethrin Mixtures as House Fly Sprays[1]

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After the removal of piperine from extracts of *Piper nigrum* L., Spring & Stark (1950) isolated a second compound, to which they gave the name "piperettine." They identified it as the vinyl analog of piperine and synthesized it. Because piperine has been found to be highly synergistic when combined with pyrethrins, tests were undertaken to examine this new compound for this property with either pyrethrins or allethrin.

MATERIALS AND METHODS.—The sample of piperettine used was extracted from *Piper nigrum* and purified by Martin Jacobson of the Entomology Research Branch. In the sample of pyrethrum extract 61% of the total pyrethrins consisted of pyrethrin I and cinerin I, as determined by the mercury-reduction method. The sample of allethrin was 93% pure as determined by the hydrogenolysis method.

Stock solutions of the two insecticides were prepared by dissolving them in refined kerosene (Deobase). To insure complete solution of piperettine in its stock solution, acetone was used as an auxiliary solvent in the amount of 10% by volume. Sprays of the insecticides alone and in mixtures with piperettine were prepared from the stock solutions, the concentration of acetone in the diluted mixtures being maintained uniformly at 10%. With the exception of one spray in the preliminary tests, the mixtures were prepared only in the proportion of 10 parts of piperettine to 1 part of the insecticide.

Knockdown and mortality of laboratory-reared house flies, *Musca domestica* L., treated with these sprays were determined in duplicated tests by the Campbell turntable method. In each test approximately 100 flies were used; they averaged 2½ days in age in the preliminary tests and 2 days in the subsequent ones. In the preliminary work (series 1), reported because it furnishes considerable corroborative information, all tests were made on a single

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population of flies. In the subsequent, more critical work (series 2) all sprays were tested simultaneously on each of two populations.

To evaluate relative toxicity and determine the precision of the estimates, the mortality data of the second portion of the work were subjected to probit analysis as described by Finney (1952).

KNOCKDOWN AND MORTALITY.—Piperettine alone at 200 mg. per deciliter caused no knockdown of flies in 25 minutes. All other sprays caused complete knockdown.

The mortality data are summarized in table 1. Since piperettine alone caused no mortality at 200 mg. per deciliter, it may be assumed that, if it did evince toxicity at higher concentrations, its relative toxicity would be so low as to have practically no influence on the approximate effects sought. Therefore, a possible contribution by this compound to the expected toxicity of the mixtures is ignored.

EVALUATION OF RELATIVE TOXICITY AND INTENSITY OF SYNERGISM.—Individual provisional regression lines were fitted statistically to the mortality data in series 2 for the insecticides and the pyrethrins mixture. The regression coefficients with their standard errors were 2.52 ± 0.38 probits per unit log concentration for allethrin, 2.42 ± 0.14 for pyrethrins, and 2.83 ± 0.25 for the mixture suggesting that the data for the three materials could be fitted by parallel lines. Therefore, this was done and subsequent analysis of chi-squares gave no evidence of significant departure from parallelism or a need for a heterogeneity factor in the calculation of variances.

The final equations showing the regression of mortality, expressed in probits, on concentration in milligrams per deciliter, expressed as logarithms, are given below. The equation for the pyrethrins mixture is based on the expression of concentration in terms of pyrethrins content only. The standard error of the common regression coefficient is 0.118.

Pyrethrins+piperettine	$Y = 2.524X + 2.072$
Pyrethrins	$Y = 2.524X - 0.987$
Allethrin	$Y = 2.524X + 0.107$

From these equations median lethal concentrations (LC-50's) were estimated and relative toxicities were calculated as the inverse ratios of the relevant pairs of estimations. The results for pyrethrins and its mixtures are presented below.

	Pyrethrins	*Pyrethrins +Piperettine*
LC-50 (mg. per dl.)	235.7	14.46+144.6
Relative standard error of LC-50 (per cent)	3.69	5.14
Ratio of toxicity	1.0	13.70±0.87
Log of ratio	0	1.212
Minimum required to demonstrate synergism:		
Log	—	0.054
Ratio	—	1.13

Since the mixtures contained 10% of acetone and the insecticide standards none, the ratio obtained on the original basis (16.30) had to be adjusted for the increase in relative toxicity due to the acetone, the increase being nearly 20% for this amount. This was done simply by referring the estimates obtained to 1.19 instead of to 1 (Gersdorff 1956). The adjusted ratio of toxicity of the mixture, then, was 16.30 divided by 1.19, or 13.70.

Table 1.—Mortality of house flies caused by pyrethrum and allethrin sprays with and without piperettine.

MATERIAL	CONCENTRATION (MG. PER DL.) Insecticide	Piperettine	MORTALITY PER CENT IN 1 DAY
	Series 1		
Piperettine	—	200	0
Pyrethrins+piperettine	100	200	95.7
	25	250	83.9
	12.5	125	62.3
Pyrethrins	506	—	88.5
	225	—	65.0
	100	—	32.1
Allethrin+piperettine	25	250	22.6
Allethrin	88.9	—	74.4
	59.3	—	55.6
	39.5	—	38.0
	Series 2		
Pyrethrins+piperettine	25	250	76.1
	12.5	125	42.6
	6.25	62.5	15.9
Pyrethrins	759	—	92.5
	506	—	74.0
	338	—	63.6
	225	—	51.0
	150	—	34.1
	100	—	17.1
Allethrin+piperettine	25	250	15.3
Allethrin	88.9	—	51.4
	59.3	—	33.6
	39.5	—	19.1

In the preliminary tests the allethrin mixture caused such low toxicity that it was apparent that the synergistic effect, if present, was no more than slight. Therefore, no extended series of tests was made with this mixture to encompass an LC-50. However, an estimate of its relative toxicity was obtained by substituting the probit of the mortality caused by the mixture in series 2 of table 1 in the allethrin equation to obtain the concentration of allethrin to which it was equivalent and comparing this value with the concentration of allethrin actually in the mixture. The two values are 34.1 and 25.0, giving an estimate of toxicity relative to allethrin of 1.37 for this mixture, which when adjusted for the acetone becomes 1.15. Thus, the mixture is but 15% more toxic than allethrin alone.

The LC-50 and toxicity relative to pyrethrins found for allethrin alone were 86.87 mg. per deciliter and 2.70. Since the latter value is close to the mean of many estimates of this ratio by this method, the soundness of the sample of pyrethrins is demonstrated.

CONCLUSIONS.—The results clearly establish piperettine as a synergist for fly sprays. The intensity of synergism with pyrethrins was very high, the toxicity of the mixture being 14 times that expected. This intensity places the compound, as appraised by the turntable method, among the best commercial synergists with pyrethrins, such as sulfoxide (Gersdorff *et al.* 1952), sulfone (Gersdorff

et al. 1955), and piperonyl butoxide (Gersdorff *et al.* 1951). With allethrin, however, the intensity of synergism was very low. Piperettine was no more, and probably less, effective in synergizing allethrin than the three commercial synergists named above.

Knockdown of flies was complete in all sprays containing the insecticides. Piperettine alone at 200 mg. per deciliter caused no knockdown or mortality.

REFERENCES CITED

Finney, D. J. 1952. Probit analysis, 318 pp. Cambridge.

Gersdorff, W. A. 1956. Effect of acetone on toxicity in pyrethrum and allethrin space sprays. Jour. Econ. Ent. **49**(6): 849–51.

Gersdorff, W. A., Norman Mitlin, and R. H. Nelson. 1955. The comparative effect of a sulfone in pyrethrum and allethrin mixtures as house fly sprays. Jour. Econ. Ent. **48**(1): 9–11.

Gersdorff, W. A., R. H. Nelson, and Norman Mitlin. 1951. The relative effect of several pyrethrum synergists in fly sprays containing allethrin. Jour. Econ. Ent. **44**(6): 921–7.

Gersdorff, W. A., R. H. Nelson, and Norman Mitlin. 1952. Sulfoxide and 3,4-methylenedioxybenzyl *n*-propyl ether in pyrethrum and allethrin mixtures as house fly sprays. Jour. Econ. Ent., **45**(6): 905–8.

Spring, F. S., and James Stark. 1950. Piperettine from *Piper nigrum*; its isolation, identification, and synethsis. Jour. Chem. Soc. (London) **1950**(2): 1177–80.

Systemic Effect of Dipterex on the Bed Bug and Gulf Coast Tick when Administered to Rabbits[1]

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Experiments have been conducted at the Agricultural Experiment Station of the Alabama Polytechnic Institute during the past several years to find chemicals relatively non-toxic to warm-blooded animals when administered orally but lethal to bloodsucking ectoparasites feeding on the animals. Of 13 chemicals tested (Adkins *et al.* 1955), Dipterex was considered one of the most promising. Additional research conducted with this material is herein reported.

MATERIALS AND METHODS.—Studies were conducted to determine the toxicity of Dipterex to fifth-instar nymphs of the bed bug, *Cimex lectularius* L., and nymphs of the Gulf Coast tick, *Amblyomma maculatum* Koch, which fed upon domestic rabbits dosed orally with the chemical. The technical material of 99% purity was administered to the animals in a water solution.

Procedure with Bed Bugs.—A randomized block design involving five dosage levels was used in the experiments. The doses were: 0, 10, 20, 30, and 40 mg. of the chemical per kg. of body weight of the rabbit. The chemical was administered orally with a hypodermic syringe to the rabbits through a stomach tube (Davol 8 Fr. urinary catheter). The 0 mg./kg. level or control was replicated four times and consisted of bugs that fed on the rabbits in the first replication before they were dosed. All other levels were replicated seven times. Two experiments were conducted. In the first experiment, two groups of 10 bugs were allowed to feed on the shaved abdomen of each rabbit. The first group began feeding 1 hour after the rabbits were dosed and the second group, 2 hours after. All bed bugs used in the experiments were allowed to take one blood meal. Counts of live and dead bugs were made every 24 hours after feeding for 6 days, at which time the living bugs molted. The last counts were used in the statistical analysis. To test the effect of a second identical dose of the systemic, the experiment was repeated 2 weeks later, using the same rabbits and the same procedures, except that the number of bed bugs per group was increased to 25.

In the statistical analysis, all percentages of mortality were converted to angles.[3] The LD-50 value (angle 45) was calculated by plotting angle mortality of bed bugs against dosage of the chemical to the rabbits using linear regression methods. In the analysis of variance and the linear regression computations, the 0 and 40 mg./kg. rates were omitted in order that only the linear portion of the mortality response was utilized (Bliss 1952).

Procedure with Ticks.—In conducting experiments with ticks, a randomized block design was again used involving 26 rabbits. Five dosage levels were administered to the rabbits orally through a stomach tube (Bardam 12 Fr. curved tip urinary catheter). These dosage levels were: 0, 25, 50, 75, and 100 mg. of chemical per kg. of body weight of rabbit. Each dose was measured with a burette into a glass hypodermic syringe attached to the stomach tube. The 0 mg./kg., or control, was replicated two times and all other dosage levels were replicated six times. Two days before the rabbits were dosed, from 40 to 60 nymphal ticks were introduced into each of the feeding chambers. These chambers had been previously glued and taped to the clipped backs of the rabbits. The number of ticks that attached to each rabbit varied from 6 to 41 and averaged 20.7 per rabbit. Counts of dead and live ticks were made 24 hours after the rabbits were dosed and again at the time when the living ticks engorged and dropped from the hosts. The latter count was used in the statistical analysis.

In preliminary tests there was some evidence that the sex of the dosed rabbits on which the ticks were feeding influenced the mortality of the ticks. To test this interaction of the effect of the rabbits' sex with dose, the experiment was designed so that three rabbits of each of the

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[3] Angle = Arc sin $\sqrt{\text{proportion}}$ (Snedecor 1956).

Table 1.—Average mortalities of fifth-instar bed bugs after feeding on rabbits dosed with Dipterex.

Dose to Rabbits (Mg./Kg.)	Number of Rabbits Used	Number of Bugs Fed on Rabbits	Per Cent Mortality of Bed Bugs on Dosed Rabbits: 1-Hr. After Dosing	2-Hrs. After Dosing
		First Experiment		
0	4	40	0	0
10	7	70	54.3	10.0
20	7	70	78.6	52.9
30	7	70	85.7	84.3
40	7	70	100.0	100.0
		Second Experiment		
0	4	100	0	0
10	7	175	34.9	41.1
20	7	175	86.3	69.7
30	7	175	87.4	79.4
40	7	175	93.7	100.0

six replicates were of the opposite sex. Thus, there was a total of three replicates of each sex.

In the statistical analysis, all percentages of mortality were converted to angles. The LD-50 value (angle 45) was calculated by plotting angle mortality of ticks against dosage of the chemical to rabbits using linear regression methods. In the analysis of variance and the linear regression computations, the 0 and 100 mg./kg. rates were omitted in order that only the linear portion of the mortality response was utilized.

RESULTS AND ANALYSES.—*Bed Bugs.*—The average mortality percentages of bed bugs that fed upon rabbits in the first and second experiments are presented in table 1. In experiment 1 each percentage mortality represents 70 bugs that fed on 7 rabbits. In experiment 2 each percentage mortality represents 175 bugs that fed on 7 rabbits.

Results of the statistical analysis indicated that there was a highly significant difference between the dose levels. Although there was no statistical significance due to replicates (groups of rabbits), the mean square for replications was more than twice the mean square for error. This probably indicated that groups of rabbits could have variability, which would be removed from experimental error by the device of blocking used. The results also indicated that response of the rabbits to dose levels in the second experiment was significantly different

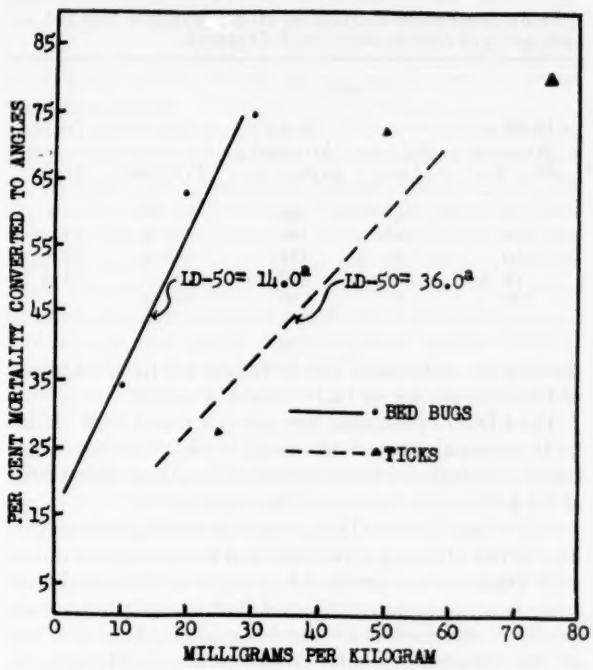

FIG. 1.—Regression of angle mortality of bed bugs and Gulf Coast ticks on mg. of Dipterex per kg. body wt. of rabbit.

[a] LD-50 = Angle 45.

from the first. The data indicated that this response was probably due to random factors that would not be repeated in the same pattern in further cases of testing the effect of second doses. Therefore, data from the two experiments were pooled. Results are presented in table 2. Each mortality datum represents the mortality of 14 groups of bugs consisting of 245 bugs per dosage level.

When the data were pooled, results of the statistical analysis indicated that there was no significant difference in the angle mortality of the bugs that began to feed 1 hour after the rabbits were dosed and those bugs that began to feed 2 hours after the rabbits were dosed. Therefore, the groups of bugs that began feeding 1 and 2 hours after the rabbits were dosed were pooled. The data are presented in the last column of table 2. In these pooled data, each mortality datum represents 28 groups of bugs consisting of 490 bugs per dosage level. The mortality ranged from 36% on rabbits receiving 10 mg. of Dipterex per kg. of body weight to 98% on rabbits dosed at the rate of 40 mg./kg. The regression of the angle kill on the dosage in mg./kg. is presented in figure 1,

Table 2.—Pooled mortalities of fifth-instar bed bugs in both experiments after feeding on rabbits dosed with Dipterex.

Dose to Rabbits (Mg./Kg.)	Number of Rabbits Used	Number of Bugs Fed on Rabbits at Each Feeding	Mortality of Bed Bugs Fed on Dosed Rabbits: Per Cent 1-Hr. After Dosing	Per Cent 2-Hrs. After Dosing	Per Cent 1-Hr. and 2-Hrs. After Dosing	Angle 1-Hr. and 2-Hrs. After Dosing
0	8	140	0	0	0	—
10	14	245	40.4	32.2	36.3	34.1
20	14	245	84.1	64.9	74.5	63.7
30	14	245	86.9	80.8	83.9	75.2
40	14	245	95.5	100.0	97.8	—

Table 3.—Average mortalities of tick nymphs that fed on male and female rabbits dosed with Dipterex.

Dose to Rabbits (Mg./Kg.)	Male Rabbits: Number of Ticks	Male Rabbits: Per Cent Mortality	Female Rabbits: Number of Ticks	Female Rabbits: Per Cent Mortality
0	27	0	27	0
25	85	21.2	46	32.6
50	95	93.7	76	81.6
75	59	94.9	53	98.1
100	39	100.0	51	100.0

Table 4.—Pooled mortalities of tick nymphs that fed on both sexes of rabbits dosed with Dipterex.

Dose to Rabbits (Mg./Kg.)	Number of Rabbits Used	Number of Ticks Attached to Rabbits	Mortality at Dropping Time: Per Cent	Mortality at Dropping Time: Angle
0	2	54	0	—
25	6	131	25.2	27.8
50	6	171	88.3	72.0
75	6	112	95.5	81.4
100	6	90	100.0	—

showing the calculated LD-50 (angle 45) to be 14.0 mg. of Dipterex per kg. of body weight of rabbit.

The LD-50's presented here are not true LD-50 values to the ectoparasites in the usual sense. The values are doses, administered to the rabbits. These doses killed 50% of the arthropods that fed on the rabbits.

Gulf Coast Ticks.—The average percentage of mortalities of ticks feeding upon male and female rabbits dosed with Dipterex are presented in table 3. The statistical analysis of these data indicated that mortalities by dose levels of attached ticks were not affected by the sex of the treated rabbits (non-significant interaction). Further, there was no general difference in response level of the sexes, so that average mortalities of ticks feeding on both male and female rabbits were used to evaluate doses (table 4). Dose levels were significant, and differences between replicates (blocks of rabbits) were found. The mortality of ticks ranged from 25% on rabbits receiving 25 mg. of Dipterex per kg. of body weight to 100% on rabbits dosed at the rate of 100 mg./kg. The regression of the angle mortality of the ticks on the dosage in mg./kg. is shown in figure 1. The LD-50 (angle 45) was calculated to be 36.0 mg. of Dipterex per kg. of body weight of rabbit.

SUMMARY.—Replicated experiments were conducted to determine the dosage response of Dipterex to fifth-instar nymphs of the bed bug, *Cimex lectularius* L., and nymphs of the Gulf Coast tick, *Amblyomma maculatum* Koch, which fed upon rabbits dosed orally with the chemical. Mortalities of bed bugs after feeding were: on rabbits receiving 0 mg./kg. of body weight, 0% mortality; 10 mg./kg., 36.3%; 20 mg./kg., 74.5%; 30 mg./kg., 83.9% and 40 mg./kg., 97.8%. Mortalities of ticks were: on rabbits receiving 0 mg./kg., 0% mortality; 25 mg./kg. 25.2%; 50 mg./kg., 88.3%; 75 mg./kg., 95.5%; and 100 mg./kg., 100%. Dipterex given to rabbits 1 or 2 hours before the bugs fed killed 50% of the bed bugs at a dosage level of 14.0 mg./kg. The LD-50 to the ticks through the rabbit was calculated to be 36.0 mg./kg. body weight of rabbit.

REFERENCES CITED

Adkins, T. R. Jr., W. L. Sowell, and F. S. Arant. 1955. Systemic effect of selected chemicals on the bed bug and lone star tick when administered to rabbits. Jour. Econ. Ent. 48(2): 139–41.

Bliss, C. I. 1952. The Statistics of Bioassay. Academic Press Inc. 1st edition: p. 452.

Snedecor, G. W. 1956. Statistical Methods. The Iowa State College Press. 5th edition: p. 316.

Role of Various Insects and Mites in the Transmission of Wheat Streak-Mosaic Virus[1]

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The most prevalent and destructive virus disease of winter wheat in the Great Plains region is wheat streak-mosaic. This disease has occurred sporadically, and at times has been responsible for complete failure of the winter wheat crop is some localities. Attention was focused on it from 1947 through 1953 because of both widespread and localized epidemics in western Kansas and Nebraska and eastern Colorado and Wyoming.

According to Haskell & Wood (1923), Peltier in Nebraska transferred unidentified aphids feeding on "mosaic wheat plants" in the field to corn seedlings in the greenhouse and produced a "slight infection" on one of the test plants. McKinney (1937) also reported that he had transmitted a "Kansas wheat mosaic" by means of an unidentified aphid. Atkinson (1949) claimed that the greenbug, *Toxoptera graminum* (Rond.), was the vector of western wheat mosaic in Colorado. Wheat streak-mosaic is now known to be the same as wheat mosaic, the name employed by Atkinson to differentiate it from the soil-borne virus disease then thought to exist only east of the Mississippi River. Slykhuis (1951) in South Dakota reported that occasionally test plants became infected with wheat streak-mosaic when the leafhoppers *Exitianus exitiosus* (Uhler) and *Aceratagallia sanguinolenta* (Prov.) which had fed previously on a virus source were placed on them. However, he considered that such infection occurred accidentally. Later Slykhuis (1953) in Alberta, Canada, implicated the wheat curl mite, *Aceria tulipae* (Keif.), as the vector of this virus.

Painter (1950) in Kansas stated that "Tests of possible transmission of streak-mosaic of wheat by seven arthropod species, involving hundreds of individuals, so far have

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been unsuccessful." Harvey (1951) conducted 481 transmission tests and obtained mosaic symptoms in nine of them. He believed that leafhoppers were possibly the vector. Melchers *et al.* (1953) reported that "Eight hundred thirty-four vector tests of various wheat-feeding insects were made. These tests involved more than 30 species and thousands of individuals. At least 50 cases of transmission are considered doubtful, due to lack of equipment of the type necessary to prevent contamination."

From 1951 to 1953 the writers made studies in Kansas and Nebraska to ascertain the vector of wheat streak-mosaic virus and to clarify the status of vectors previously reported.

EXPERIMENTAL WORK.—Various methods were employed in these tests. In some of them the insects were removed from diseased wheat plants and confined by means of Lumite screen cages to pots containing five or six healthy wheat seedlings. In others the insects, after having fed on diseased plants for various lengths of time, were introduced to pots of healthy seedlings in a special insect-proof cage described by Fellows & Connin (1952). In many of the tests the insects were removed to individual healthy seedlings under lamp chimneys. Generally the test plants were kept in the greenhouse under normal conditions with temperatures ranging from 75° to 85° F. However, in some tests these conditions were altered to determine whether such changes might affect disease transmission.

The results obtained in most of these transmission experiments are summarized in table 1.

Aphids.—When the wheat streak-mosaic problem became prominent in 1946, greenbugs were very destructive to wheat in the epidemic areas. It was therefore inevitable that greenbugs should be thought connected with the transmission of this virus. The assertion of Atkinson (1949) to this effect was strengthened by the work of Ingram & Summers (1936, 1938), who reported that this aphid transmitted sugarcane mosaic virus. However, as indicated in table 1, the writers' attempts to incriminate greenbugs as vectors of wheat streak-mosaic virus were unsuccessful. Several of the tests were conducted at 45° to 55° F. to see if the reduced temperatures would predispose healthy wheat plants to infection by green bugs.

The aphid *Rhopalosiphum subterraneum* (Mason) usually infests the roots and crowns of newly emerged wheat in the fall throughout Kansas and Nebraska. Its abundance each autumn made this species suspected as a vector of wheat streak-mosaic virus, although its summer host plants on the Great Plains are unknown. Although again temperatures were reduced to 45° to 55° F. in some of the tests, only negative results were obtained.

The corn leaf aphid, *Rhopalosiphum maidis* (Fitch), migrates from corn and sorghum fields to the wheat crop during the autumn. The less numerous English grain aphid, *Macrosiphum granarium* (Kby.), is also found on the wheat crop in the fall and again in early spring. The apple grain aphid, *Rhopalosiphum fitchii* (Sand.), on the contrary, is found in greatest abundance in wheat fields in the spring. None of these species occur in large numbers, and it was improbable that they were vectors of wheat streak-moasic virus. All attempts at transmitting the virus with these aphids were negative.

Six tests were conducted with the aphid *Macrosiphum dirhodum* (Walker), because of its ability to transmit the barley yellow dwarf virus in California (Oswald & Huston 1953), although this species had not been reported infesting wheat. The results indicate that this species is not a vector of wheat streak-mosaic virus.

Leafhoppers.—The most common species of leafhoppers in wheat fields during the fall are the six-spotted leafhopper, *Macrosteles fascifrons* (Stål), and the painted leafhopper, *Endria inimica* (Say). No evidence was obtained that the six-spotted leafhooper was a vector of wheat streak-mosaic virus. However, 16 infected plants were obtained when the painted leafhopper was employed, the primary symptoms appearing in 16 to 29 days. The test plants were maintained at 75° to 85° F. At these temperatures the initial symptoms appear in 5 to 7 days (Sill & Fellows 1953). This suggests that the 16 cases of transmission are explicable only on the basis of accidental contamination or the presence of the true vector which escaped notice. A number of additional tests to implicate the painted leafhopper as a vector were completely negative. It can only be surmised, therefore, that the painted leafhopper is not a vector.

Single tests with each of three leafhopper species that are uncommon on wheat—*Cuerna gladiola* Om. & B, *Commellus* sp., and *Empoasca* sp. gave negative results.

Thrips.—Various species of Thysanoptera are common on wheat, and at least two unidentified species were tested as vectors of wheat streak-mosaic virus with negative results.

Grasshoppers.—Grasshoppers sometimes feed at the margins of winter wheat fields in the fall. Occasionally a field is found in which the number of plants infected by the wheat streak-mosaic virus is greatest along one of the margins. Accordingly tests were conducted with the migratory grasshopper, *Melanoplus mexicanus* (Sauss.), and the two-striped grasshopper, *M. bivittatus* (Say). All the results were negative.

MISCELLANEOUS INSECTS.—Negative results were obtained with the chinch bug, *Blissus leucopterus* (Say),

Table 1.—Results of tests on the transmission of wheat streak-mosaic with various wheat insects.

INSECT[a]	NUMBER OF INSECTS PER TEST	DAYS THAT INSECTS WERE KEPT ON— Virus Source	Test Plants	DAYS THAT TEST PLANTS WERE OBSERVED	RATIO OF TESTS SHOWING TRANSMISSION TO TOTAL NUMBER OF TESTS
Blissus leucopterus	5	7	16	30	0/4
Chactocnema pulicaria	15	7	10	30	0/1
Commellus sp.	5	5	14	30	0/1
Cuerna gladiola	8	8	14	30	0/1
Empoasca sp.	7	7	5	45	0/1
Endria inimica[b]	1–20	3–24	3–14	33–52	16/165
Macrosiphum dirhodum	25	—[c]	9	48	0/6
Macrosiphum granarium	3–10	1–21	9–10	41–60	0/45
Macrosteles fascifrons[b]	3–17	2–14	2– 7	31–42	0/31
Melanoplus bivittatus	10–15	1–14	4–14	40–55	0/4
Melanoplus mexicanus	8–15	2–14	4–14	40–45	0/5
Meromyza americana	3–50	2– 8	2– 9	30–55	2/30
Phalacrus sp.	2– 7	4	5–14	30–60	1/5
Rhopalosiphum fitchii	5–25	1–38	7–14	29–41	0/173
R. maidis	3–50	1–50	3–34	30–60	0/174
R. subterraneum	1–12	1–58	2–11	30–72	0/131
Thysanoptera	3– 8	1–38	3–30	30–90	0/45
Trigonotylus ruficornis	10	7	7	40	0/3
Toxoptera graminum	1–50	1–20	2–16	30–63	0/253

[a] Adults unless otherwise indicated.
[b] Adults and nymphs.
[c] Reared on infested plants.

Table 2.—Results obtained in the transmission of wheat streak-mosaic virus with the wheat curl mite.

Source of Mites and Virus	Number of Mites per Test Plant	Ratio of Tests Showing Transmission to Total Number of Tests
Reared on manually inoculated wheat in greenhouse at Manhattan, Kans.	16–50	7/7
Reared on manually inoculated wheat in greenhouse at Lincoln, Nebr.	5–25	19/20
Mites and diseased wheat received from J. T. Slykhuis, Alberta, Canada	2–50	9/12
Infected field wheat, Kansas	8–10	4/4
Infected wheat, mosaic nursery, Manhattan, Kans.	2–50	19/21
Infected field wheat, Nebraska	1–10	8/14

the leaf bug *Trigonotylus ruficornis* (Geoffrey), and the corn flea beetle, *Chaetocnema pulicaria* Melsh. However, when the beetle *Phalacrus* sp. was used, infected plants were produced in one test, although the results could not be duplicated. In the test in which infection took place, the initial symptoms did not develop until 25 days after the insects had been removed from the test plants. Infection in these plants was likely caused by contamination, either mechanically or from the presence of the true vector.

In 2 of 30 tests with the wheat stem maggot, *Meromyza americana* Fitch, test plants also became infected. Again the appearance of initial symptoms 45 days after the insects had been taken from the test plants indicates contamination.

Acarina.—The brown wheat mite, *Petrobia latens* (Müll.), occasionally appears in numbers in wheat fields in the late spring. A single test with this species indicated that it was not a vector of wheat streak-mosaic virus.

With the announcement by Slykhuis (1953) that the wheat curl mite was the vector of wheat streak-mosaic virus, 78 tests to transmit the virus with this mite were made. The experimental conditions and the results obtained in these tests are presented in table 2. It is quite apparent that this mite is a vector. No matter what the source of the mites, whether reared on manually inoculated plants in the greenhouse or obtained from infected plants in the field, this species was capable of transmitting the virus. Single viruliferous mites caused infection, and in all tests initial symptoms developed in 5 to 7 days. When manual inoculations were made with leaves from infected test plants as sources of inocula, the positive results demonstrated that the mites had transmitted the virus and that the symptoms on the plants were not toxicogenic effects. Staples & Allington (1956) and Connin (1956), in studying the epidemiology of wheat streak-mosaic in Nebraska and Kansas, respectively, have shown the relationship of this mite to the transmission of wheat streak-mosaic virus in the field.

The wheat curl mite is very small, approximately 220 microns in length in the adult stage, and not readily discernible with the naked eye. It can easily pass through screen and cloth barriers that confine insects as small as thrips. Consequently, cases of transmission of wheat streak-mosaic virus reported in the literature could have resulted from the unknown presence of this eriophyid, particularly when diseased wheat collected in the field was used as the virus source for the test insects. Wheat curl mites are often seen clinging to the legs of thrips and aphids as they progress along a wheat leaf heavily infested with mites. Insects carrying their mite burden from an infected plant to healthy test plants could result in infection. The wheat curl mite is easily picked up on the transfer needle or brush. Slight movements of the air can also transport them.

SUMMARY.—From 1951 through 1953 studies were made in Kansas and Nebraska to ascertain the vector of wheat streak-mosaic virus and to clarify the status of vectors previously reported. Most of the common insects and mites and some of the more uncommon ones found on wheat in Kansas and Nebraska did not transmit this virus. However, its transmission by the wheat curl mite, *Aceria tulipae* (Keif.), was confirmed. This mite is probably the sole vector of the virus in the Great Plains region.

REFERENCES CITED

Atkinson, R. E. 1949. Western wheat mosaic in Colorado and its transmission by the grain aphid, *Toxoptera graminum*. Phytopath. **39**(1): 2.

Connin, R. V. 1956. Oversummering volunteer wheat in the epidemiology of wheat streak-mosaic. Jour. Econ. Ent. **49**: 405–6.

Fellows, H., and R. V. Connin. 1952. A glass and plastic insect-proof cage. Plant Dis. Reporter **36**(12): 471–3.

Haskell, R. J., and J. I. Wood. 1923. Diseases of cereal and forage crops in the United States in 1922. Plant Dis. Reporter Supp. **27**: 164–226.

Harvey, T. L. 1951. Transmission experiments involving possible insect vectors of the virus, *Marmor virgatum* var. *typicum* McKinney, which causes wheat yellow streak mosaic. Unpublished M.S. thesis, Kansas State College.

Ingram, J. W., and E. M. Summers. 1936. Transmission of sugarcane mosaic by the rusty plum aphid. Jour. Agric. Res. **52**(11): 879–87.

Ingram, J. W., and E. M. Summers. 1938. Transmission of sugarcane mosaic by the greenbug (*Toxoptera graminum* Rond.). Jour. Agric. Res. **57**(7): 537–40.

McKinney, H. H. 1937. Mosaic diseases of wheat and related cereals U. S. Dept. Agric. Circ. **442**.

Melchers, L. E., R. C. Smith, and R. V. Olson. 1953. Virus diseases of hard red winter wheat. Kansas Agric. Expt. Sta., 16th Rept.

Oswald, J. W., and B. R. Houston. 1953. The yellow-dwarf virus of cereal crops. Phytopath. **43**(3): 128–36.

Painter, R. H. 1950. Hessian fly and other wheat insects. Kansas Agric. Expt. Sta., 15th Rept. 1948–50.

Sill, W. H., Jr., and H. Fellows. 1953. Symptom expression of the wheat streak-mosaic disease as affected by temperature. Plant Dis. Reporter **37**(1): 30–3.

Slykhuis, J. T. 1951. The relation of leafhoppers to certain symptoms on wheat in South Dakota. Plant Dis. Reporter **35**(10): 439–40.

Slykhuis, J. T. 1953. Wheat streak-mosaic in Alberta and factors related to its spread. Canadian Jour. Agric. Sci. **33**: 195–97.

Staples, R., and W. B. Allington. 1956. Wheat streak mosaic in Nebraska and its control. Nebraska Agric. Expt. Sta. Res. Bull. **178**.

Fig Scale Parasites Introduced into California[1]

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The establishment in California of the natural enemies of the fig scale, *Lepidosaphes ficus* (Sign.), has been a project of the Department of Biological Control in the University of California for the past 18 years. The fig scale however has existed in California since 1905 when it was introduced into Fresno County by means of fig cuttings from Algeria. The natural dispersion of the scale was very slow. At the end of 10 years, less than 100 trees were heavily infested, these being the trees that had been grafted with scions from the imported cuttings. The entire infestation was limited to an area having a radius of less than a mile (Roullard 1917).

In the subsequent years the natural dispersion of the fig scale appeared to be greatly accelerated artificially by the distribution of infested caprifigs. This dispersion closely followed the expansion of caprifig and Smyrna fig plantings initiated by the successful introduction of the fig wasp, *Blastophaga psenes* (L.), in 1899.

By 1937 the Merced County Agricultural Commissioner, C. H. Kinsley, considered the fig scale the most important enemy of the fig crop. It then occurred in all the fig growing sections of Fresno and Tulare counties, as well as in the areas around Merced and Modesto. Stafford & Barnes (1948) noted that in Merced County it attacked all the varieties of figs. It has been found also on the elm, walnut, and rose.

The fig scale, after its establishment in California, was attacked by natural enemies that were already present and subsisting on other species of scale insects. These natural enemies included the ladybird beetles *Lindorus lophanthae* (Blaisd.) and *Chilocorus bivulnerus* Muls., several species of lacewings, and the predatory mites *Hemisarcoptes* sp. and *Seiulus* sp. Stafford & Barnes (1948) studied the predator-caused mortality of the fig scale populations during the fall and winter of 1944–45 and found it to be about 66%. They also observed that the ectoparasite, *Aphytis mytilaspidis* (LeBaron), although present, was of no importance since it destroyed only 1.4% of the large population of scale that had survived the attack of the predators. The work of an undetermined endoparasite was observed by the writer in collections of fig scale made in the vicinity of Stockton in 1939. The total action of these accrued natural enemies, however, was not sufficient to prevent the scale attaining damaging proportions. Consequently, the addition of natural enemies from the original habitat of the scale became imperative.

Foreign Introductions.—In 1939 the University of California began the introduction of natural enemies from the Mediterranean regions. A series of these introductions took place during a period of 16 years.

During the first 6 months of 1939, Harry S. Smith visited Italy in order to obtain the natural enemies of certain scale insects including the fig scale. Early in May he discovered that the fig scale was parasitized by the chalcidoid, *Physcus testaceous* Masi, that also attacked the oyster-shell scale, *Lepidosaphes ulmi* (L.). In July he reared several hundred adults of *Physcus* from oyster-shell scale and sent them to California. Only 80 females survived shipment. Some of these had mated before the males died.

Smith (1939) considered that the establishment of this *Physcus* in California promised to be the most important result of his trip to Italy since there he found it commonly parasitizing the oyster-shell scale and the fig scale. Only on the fig did this parasite appear to give effective control.

Since the oyster-shell scale and fig scale are difficult to propagate under the artificial conditions of the laboratory and since it is the policy of the University of California not to release natural enemies until they are known to be entirely beneficial in habit, the female *Physcus* received from Smith were used only in biological studies. These studies, using the oyster-shell scale as a host, showed that *Physcus testaceous* oviposits in the half-grown scale and emerges as an adult from the full-grown scale, that the adults thus obtained are all females, and that these females are the only progeny of the mated females. The male of the *Physcus*, as in certain other Aphelinids, is produced only as the progeny of the unmated female. The unmated female oviposits ectoparasitically as a secondary parasite of various diaspine scale insects. The hosts of the ectoparasitic male appear to include only the full-fed larvae of the primary parasites of various scale insects.

The mated females from Italy produced female progeny in oyster-shell scale and the unmated females produced male progeny on the larvae of *Comperiella bifasciata* Comp. in the yellow scale, *Aonidiella citrina* (Coq.). The usual host of the *Physcus* male is the larval *Physcus* female.

After it was demonstrated that *Physcus testaceous* reproduces *as a species* only as a primary parasite of *Lepidosaphes ulmi* and *L. ficus*, arrangements were made with Prof. F. Silvestri, head of the biological control work in Italy, to make additional shipments. Consequently, in 1940 two such shipments were received in February, one in May and one in June. These shipments yielded a total of 186 females and 10 males of *Physcus*.

Colonizations totalling 84 females and 21 males were made on a heavily infested dooryard tree at Clovis in Fresno County, although the stages of the scales suitable for the development of the parasite were scarce. The parasite was not recovered.

In 1947 Silvestri sent another shipment of parasitized fig scale from Italy. This shipment yielded only one female of *Physcus* and several females of *Aphytis mytilaspidis*. The female *Physcus* was allowed to oviposit on the larvae of *Comperiella* and then was placed at a cool temperature and held there until her male progeny had emerged. However, when placed with the male, the female refused to mate, apparently having lost the mating instinct while in storage.

Because of the failure to propagate *Physcus testaceous*

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in numbers under laboratory conditions, it was decided to make direct releases of adults reared from parasitized scale collected in Europe. Arrangements were made with the U. S. Department of Agriculture for shipments of such material through its European agents.

The first shipment, consisting of infested elm and fig twigs collected in Italy, was received in April, 1948, and yielded a number of parasitic Hymenoptera as follows: 910 females of *Archenomus* sp., 885 females and 25 males of *Physcus testaceous*, 46 females of *Aphytis mytilaspidis* and 29 females of *Aspidiotiphagus* sp.

Seven hundred of the *Physcus* females from this shipment were sent to the Merced County Agricultural Commissioner, E. A. Danison, who colonized them in a plot which included large populations of oyster-shell and fig scales in close association. Establishment is most likely to occur in such a situation since the life histories of the parasite and of these scales indicate that the fig scale and the oyster-shell scale serve as alternate hosts of the parasite and thus enable it to survive.

Shipments during 1949 yielded about 1,000 female *Archenomous*, over 679 *Physcus* females, over 63 females of *Aphytis mytilaspidis*, and 19 females of *Aspidiotiphagus*.

In 1950 two shipments of fig scale from France yielded 49 female *Physcus* and 6 female *Aphytis*, whereas two shipments of oyster-shell scale, collected at about the same time, yielded 2855 *Physcus* and 43 *Aphytis*.

As in previous shipments, a marked difference occurred in the sex ratios of *Physcus*. The sex ratio of *Physcus* in the shipment of elm scale collected May 6 at Elizabethville, France, was 2 females to 1 male, whereas in the shipment of elm scale collected May 10 at Nimes it was 270 females to 1 male. The ratio in the average shipment was about 25 females to 1 male.

It is probable that the failure to establish *Physcus testaceous* is an effect of inadequate colonizations, inadequate not in numbers but in timing. Colonizations of mated and unmated females should be synchronized with the occurrence of host stages suitable for the development of the male and the female and made under conditions suitable for continued reproduction.

All attempts to propagate *Archenomus* sp. failed apparently because it may be a specific parasite of the oyster-shell scale and a biparental in reproduction with hyperparasitic males, the latter being extremely scarce.

Aphytis mytilaspidis.—In 1949 it was decided to colonize on fig scale the imported form of the ectoparasite, *Aphytis mytilaspidis*, since it was available in the shipments of *Physcus* and could be propagated in large numbers on latania scale, *Aspidiotus lataniae* Sign. as had been demonstrated in 1948.

The decision to colonize *Aphytis mytilaspidis* although identified as a species already present in the fig orchards, was based on the possibility that the biological characteristics of the imported form were favorable for the reduction of the host scale. In most previous shipments this parasite had been reared from fig scale. Consequently, on March 7, 1949, several hundred adult females of *Aphytis mytilaspidis* were sent to the Albany staff of the Department of Biological Control with the request that they be released on the fig scale in the San Joaquin Valley. Dr. R. L. Doutt reported (*in litt.*) that the parasites were received in excellent condition and that they were released as directed. Although they had to be released under the worst possible conditions of wind and of rain, they showed interest in the host immediately upon release and began probing and ovipositing. The colonized females were observed ovipositing 6 days after their release. Additional material sent to Albany was colonized by Doutt as follows: 300 on Calimyrna fig tree at Fresno and 125 on Calimyrna fig tree at Merced. The first generation developing in the field from released material was recovered April 22.

On May 10th Doutt reported that the previous day he had found adult females of *Aphytis mytilaspidis* active on the branches of the fig at Merced, that on one twig there were numerous scales each with an exit hole of the parasite. During the July he found a second parasite generation developing on the very small individuals of the summer host generation, indicating that they could survive the most unfavorable period with respect to the availability of suitable host stages.

Finally, Doutt (1954) reported the definite establishment of the European form of *Aphytis mytilaspidis*, that it survived the summer in small numbers on fig scale infesting the branches and twigs of the tree and had become an important factor in the control of the scale. Samples of fig scale collected at colonization sites in 1954 showed parasitization ranging from 67% to 100%.

In 1955 a survey by Huffaker (1956) in Fresno County showed that the parasite had spread from the vicinity of Herndon to that of Clovis.

Abandoned fig trees in the vicinity of Merced were examined by the writer in June, 1956 and found to be practically free of fig scale—a remarkable contrast to the infestation that he had observed in the same area from 1939 to 1949.

If *Aphytis mytilaspidis* eventually proves to be generally incapable of giving satisfactory control of the fig scale, the attempts to establish *Physcus testaceous* should be continued not only with the form from the oyster-shell scale, so far the only form colonized to any extent, but with the form which is known to parasitize fig scale in Europe.

CONCLUSION.—A series of attempts to establish in California the chalcidoid, *Physcus testaceous*, for the control of the fig scale, *Lepidosaphes ficus*, led, in 1949, to the introduction and establishment of a European form of *Aphytis mytilaspidis*. This form of *A. mytilaspidis* caused a marked reduction in the density of the fig scale in certain orchards.

REFERENCES CITED

Doutt, Richard L. 1954. Biological control of fig. scale. California Agric. **8**: 13.

Huffaker, C. B. 1956. Spread and activity of imported parasites of fig scale. Ann. Res. Confer. California. Fig Inst. Proc. **10**: p. 23.

Roullard, Fred P. 1917. The Mediterranean fig scale. California Monthly Bull. **6**: 246–7.

Smith, Harry S. 1939. Communication dated July 12.

Stafford, E. M. and D. F. Barnes. 1948. Biology of the fig scale in California. Hilgardia **18**: 567–98.

Sweetclover Weevil Parasitized by *Beauvaria bassiana* (Bals.) Vuill. in Minnesota[1,2]

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In recent years production of sweetclover in Minnesota has been seriously curtailed by the sweetclover weevil, *Sitona cylindricollis* Fahr. (Haws & Holdaway 1955). In the spring of 1955, an experiment designed to compare the effectiveness of several insecticides in controlling the weevil apparently was confounded by a high natural mortality of the weevils in non-sprayed plots.

Examination of the weevils collected in the experimental plots indicated that hundreds of them were invaded with *Beauvaria bassiana* (Bals.) Vuill., and the fungus was fruiting profusely on the dead insects. Tests were made in the laboratory to determine whether this fungus would infect and kill the weevil larvae and adults, or whether presence of the fungus represented a secondary infection. The results of these tests are reported here.

Review of Literature.—*Beauvaria bassiana* is known to be a parasite of insects (Benham & Mirand 1953, Lefebvre 1931, 1934, Metalnikow & Toumanoff 1928, Steinhaus 1949, Stirrett *et al.*, 1937, and Toumanoff 1933) and was reported on sweetclover weevil by Bird (1949). Lefebvre (1931, 1934) reported that this fungus caused 100% mortality of European corn borer larvae (*Pyrausta nubilalis* (Hbn.) in laboratory tests. The fungus penetrated the larvae directly, ramified through the tissues, and killed the larvae in 2 days. The dead larvae became mummified, dry, hard, brittle and consisted mostly of fungus mycelium and spores.

According to Steinhaus (1949), it is the consensus of most investigators that application to soil of fungi parasitic on insects usually has not been an effective method of biological control presumably because soil often is naturally infested with inoculum, and adding a little more inoculum has not increased mortality.

Materials, Methods and Results.—Dead adult sweetclover weevils were collected from sweetclover fields. *Beauvaria bassiana* was isolated from them, identified, and increased on potato-dextrose agar. Inoculum of the fungus produced in this way was used in experiments described here.

Test 1 (adult weevils).—Tests for pathogenicity of *B. bassiana* on adult sweetclover weevils were made in the laboratory. Twenty-five insects were placed in each of 18 petri plates that contained sterilized soil and a moist filter paper. Every 2 to 4 days fresh sweetclover leaves were put in for the weevils to feed on. The weevils in nine plates were inoculated by adding a conidial suspension of the fungus; water was poured into the nine check plates. Three inoculated and three check plates were then placed at each of the three temperatures of 10°, 15°, and 20° C. The results are recorded in figure 1. Additional tests at room temperature (23 to 25° C.) gave similar results. Even though survival in the checks was low, in every case the survival in the checks was more than twice that in the inoculated plates. However, exploratory studies made separately by M. F. Kernkamp and A. Glenn Richards in laboratories at St. Paul during the winter of 1955 indicated that infection of adult sweetclover weevils by

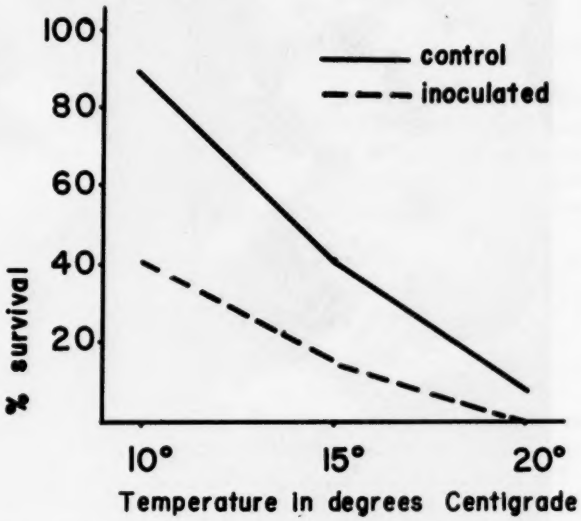

Fig. 1.—Percentage survival, at three temperatures, of adult sweetclover weevils 14 days after inoculation with *Beauvaria bassiana.*

Beauvaria did not occur at winter indoor humidities. Richards (1956) pointed out that, in contrast, the *Herpomyces* (Laboulbeniales) on cockroaches germinates freely in laboratory air which commonly falls to 20% relative humidity during the winter.

Test 2 (larvae).—Soil containing larvae was collected from a sweetclover field and taken into the laboratory in metal ointment cans (3 inches in diameter and 1 inch deep). Nineteen larvae were allowed to crawl over cultures of *B. bassiana* and then were placed in soil that had been sprayed with a spore suspension of this fugus. Twelve of the larvae collected were left in the ointment cans containing soil but these larvae were not inoculated with fungus spores. In 2 days all of the inoculated larvae were dead but all of the non-inoculated larvae were living and appeared to be in good condition.

Test 3 (larvae).—In a second experiment, 108 weevil larvae were inoculated in the same manner as described in Test 2. Ninety-six non-inoculated larvae were used as checks. Four days after the inoculation all of the inoculated larvae were dead, but all of the non-inoculated ones were living and apparently healthy.

Test 4 (larvae).—An experimental design of six replications and two treatments was used in a study of 144 other

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[2] This study has resulted from a cooperative research program on seed production of forage legumes being conducted by the University of Minnesota Agricultural Experiment Station with the assistance of the Iron Range Resources and Rehabilitation Commission.

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[4] The authors acknowledge the assistance of C. M. Christensen, Professor, Dept. of Plant Pathology and Botany, and of F. G. Holdaway, Professor, Dept. of Entomology and Economic Zoology, in this work.

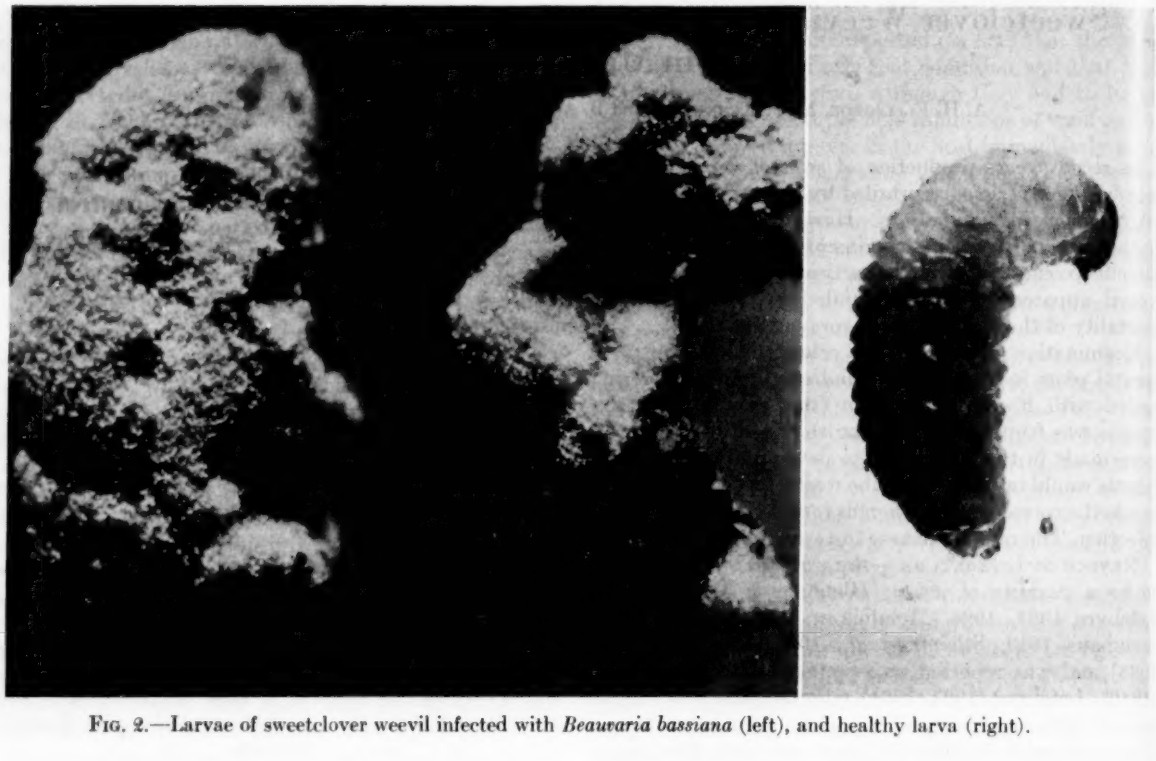

FIG. 2.—Larvae of sweetclover weevil infected with *Beauvaria bassiana* (left), and healthy larva (right).

sweetclover weevil larvae. A dozen larvae were placed in each of 12 ointment cans. Six cans of the larvae were selected at random as checks and larvae in the remaining six cans were sprayed with a suspension of fungus spores. A small amount of damp soil from which the larvae had been collected was added to each container. At the end of 10 days all 72 larvae sprayed with the spores were dead and 12 of the 72 larvae that were not sprayed were dead.

In these tests the infected larvae became sluggish and did not respond to stimuli such as light and pricking with a needle. After death, the mummies became dry and brittle. If there was adequate moisture, however, the larvae became covered with a mealy powdery fungus growth with profuse sporulation (figure 2).

CONCLUSIONS.—These tests demonstrated conclusively that *Beauvaria bassiana* infected and killed larvae of the sweetclover weevil in the laboratory, and that the fungus is capable of infecting and killing adult weevils. The results support field observations which suggested that large numbers of adult weevils were being parasitized and killed in sweetclover fields in 1955. The exact method by which weevil larvae and adults are infected by the fungus was not investigated nor were the ecological conditions most favorable for development of the fungus considered. The fungus is easily propagated on potato-dextrose agar at a temperature of 23 to 25° C.

REFERENCES CITED

Benham, Rhoda W., and J. L. Mirand. 1953. The genus *Beauvaria*, morphological and taxonomical studies of several species and of two strains isolated from wharf-piling borers. Mycologia **45**: 727–46.

Bird, R. D. 1949. Studies in the biology and control of the sweetclover weevil (Coleoptera; Curculionidae) in Manitoba, 1945–1949. Ent. Soc. Ontario 80th Ann. Rept.: 1–6.

Haws, B. A., and F. G. Holdaway. 1955. Sweetclover weevil and its control in Minnesota. Minnesota Agric. Ext. Ser. Folder 180. 8 pp. (Revised June).

Lefebvre, C. L. 1931. A destructive fungus disease of the corn borer. Phytopath. **21**: 124–25.

Lefebvre, C. L. 1934. Penetration and development of the fungus, *Beauvaria bassiana*, in the tissues of the corn borer. Ann. Botany **48**: 441–52.

Metalnikov, S., and C. Toumanoff. 1928. Experimental researches on the infection of *Pyrausta nubilalis* by entomophytic fungi. Internatl. Corn Borer Invest., Sci. Repts. **1**: 72–73.

Richards, A. Glenn. 1956. Differences in germination of *Beauvaria bassiana* (Bals.) Vuill. and *Herpomyces* (Laboulbeniales) at relative humidities in the laboratory. (Personal communication.) St. Paul. Minn.

Steinhaus, E. A. 1949. Principles of insect pathology. McGraw-Hill Book Co., Inc. New York, N. Y. 757 pp.

Stirrett, G. M., G. Beall, and M. Timonin. 1937. A field experiment on the control of the European corn borer, *Pyrausta nubilalis* Hubn., by *Beauvaria bassiana* Vuill. Sci. Agric. **17**: 587–91.

Toumanoff, C. 1933. Action des champignons entomophytes sur la pyrale du mais (*Pyrausta nubilalis*). Ann. Parasitol. Humaine et Comp. **11**: 129–44.

Repellency of Homologous Series of Cyclohexane Aliphatic Acids and Amides[1]

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Preliminary screening tests with mosquito repellents indicated that the effectiveness of a homologous series of cyclohexane aliphatic acids increased with the number of carbons in the structural chain up to a certain length and then decreased. In those tests effectiveness was based on the number of days a treated cotton stocking protected a man's arm in a cage of mosquitoes. Additional tests were conducted to determine whether the more effective compounds had a higher initial repellency or were simply retained longer on the stockings, or both, and a series of the corresponding amides was also examined. The reference code numbers and structural formulas of the compounds were as follows:

ENT No.	*Acids*	*Structural formula*
1854	Cyclohexanecarboxylic acid	$C_6H_{11}COOH$
14243	Cyclohexaneacetic acid	$C_6H_{11}CH_2COOH$
14244	Cyclohexanepropionic acid	$C_6H_{11}(CH_2)_2COOH$
12010	Cyclohexanebutyric acid	$C_6H_{11}(CH_2)_3COOH$
14245	Cyclohexanevaleric acid	$C_6H_{11}(CH_2)_4COOH$
14246	Cyclohexanehexanoic acid	$C_6H_{11}(CH_2)_5COOH$
20458	*N,N*-Diethylcyclohexanecarboxamide	$C_6H_{11}CON(C_2H_5)_2$
20837	*N,N*-Diethylcyclohexaneacetamide	$C_6H_{11}CH_2CON(C_2H_5)_2$
20827	*N,N*-Diethylcyclohexanepropionamide	$C_6H_{11}(CH_2)_2CON(C_2H_5)_2$
20816	*N,N*-Diethylcyclohexanebutyramide	$C_6H_{11}(CH_2)_3CON(C_2H_5)_2$

ACIDS.—Acetone solutions of the repellents were applied to an area of 0.3 square foot on women's mercerized-cotton stockings a short distance above the ankle portion. The stockings were hung on a line indoors to allow the solvent to evaporate and tested after intervals ranging from 2 hours to several weeks. Tests were made by drawing the stocking over the arm of a subject, with the treated portion fitting snugly over the forearm, protecting the hand with a heavy glove, and exposing the arm in a cage containing about two thousand 4-day-old *Aedes aegypti* (L.) mosquitoes. Exposure was continued for 1 minute or until more than 10 mosquitoes bit through the stocking, whichever occurred first. A stocking treated with a standard concentration of dimethyl phthalate was included in each series of tests to check the biting rate and thus assure the use of mosquitoes of high, but not excessive, avidity.

The initial repellency was determined 2 hours after treatment with several dosages of each compound. From these tests the minimum effective dosage (the minimum dosage that reduced the average number of bites per minute to less than one) was determined. The results are shown in table 1.

Increasing the length of the chain by the insertion of 1, 2, or 3 carbons increased the initial effectiveness of the repellent progressively, whereas the addition of 4 and 5 carbons decreased its effectiveness. The minimum effective dosage was 0.25 gram for cyclohexanecarboxylic acid (1854), 0.20 gram for cyclohexaneacetic acid (14243), 0.15 gram for cyclohexanepropionic acid (14244), and 0.10 gram for cyclohexanebutyric acid (12010). Cyclohexanevaleric acid (14245) and cyclohexanehexanoic acid (14246) did not provide this degree of protection at any dosage.

Residual effectiveness was determined at various intervals after treatment with a series of dosages. In order to compare the compounds at equivalent levels, the tests included a dosage of each repellent at twice the minimum effective dosage. The results are given in table 2.

The residual effectiveness at equivalent dosages also increased with the length of the chain up to a certain point. At twice the minimum effective dosage it increased through the first three repellents in the series—1854, 14243, and 14244. The fourth compound (12010) showed greater residual effectiveness to the tenth bite, but not to the first bite, than the acids with shorter chains. The fifth compound (14245) also provided protection to the tenth bite, though its initial effectiveness was not high.

The over-all increase in practical efficiency of the best

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Table 1.—Number of bites per minute by *Aedes aegypti* through stockings treated with a series of cyclohexane aliphatic acids.[a]

DOSAGE (GRAMS)	1854		14243		14244		12010		14245		14246	
	Range	Average	Range	Average	Range	Average	Range	Average	Range	Average	Range	Average
0.05	—	—	—	—	0–10+	7+	0–10+	6+	—	—	—	—
.10	—	—	0–10+	5+	0–9	3	0–0	0	3–5	4	—	—
.15	0–10+	5+	0–7	3.3	0–0	0	0–0	0	—	—	—	—
.20	0–2	1	0–0	0	0–2	0.7	0–1	0.3	1–10+	7+	—	—
.25	0–0	0	0–0	0	—	—	—	—	—	—	—	—
.30	0–0	0	—	—	0–0	0	—	—	1–8	4.3	—	—
.40	—	—	0–0	0	—	—	0–0	0	1–10+	4+	—	—
.50	0–0	0	—	—	—	—	—	—	—	—	9–10+	9+
.60	—	—	—	—	—	—	—	—	0–7	3.7	—	—
.70	—	—	—	—	—	—	—	—	—	—	7–9	8
.90	—	—	—	—	—	—	—	—	—	—	5–10+	7+
1.00	—	—	—	—	—	—	—	—	—	—	4–10+	7+
2.00	—	—	—	—	—	—	—	—	—	—	0–7	4

[a] Three replications.

Table 2.—Days stockings treated with a series of cyclohexane aliphatic acids remained effective against *Aedes aegypti*.[a]

Dosage (Grams)	1854		14243		14244		12010		14245		14246	
	Range	Average	Range	Average	Range	Average	Range	Average	Range	Average	Range	Average
Less than 1 bite per minute												
0.05	—	—	—	—	0–0	0	0–0	0	—	—	—	—
.10	—	—	0–0	0	0–0	0	0–0	0	0–0	0	—	—
.15	0–0	0	0–1	0.33	0–3	1.0	0–3	1.67	—	—	—	—
.20	0–0	0	0–1	.67	3–4	3.3	0–3	1.0[b]	0–0	0	—	—
.25	0–0	0	1–2	1.0	—	—	—	—	—	—	—	—
.30	0–1	0.33	—	—	3–4	3.67	—	—	0–0	0	—	—
.40	—	—	1–3	2.0	—	—	5–6	5.3	0–0	0	0–0	0
.50	1–1	1.0	—	—	—	—	—	—	—	—	—	—
.60	—	—	—	—	—	—	—	—	0–0	0	—	—
.70	—	—	—	—	—	—	—	—	—	—	0–0	0
.80	—	—	—	—	—	—	—	—	—	—	—	—
.90	—	—	—	—	—	—	—	—	—	—	0–0	0
1.00	—	—	—	—	—	—	—	—	—	—	0–0	0
2.00	—	—	—	—	—	—	—	—	—	—	0–0	0
Less than 10 bites per minute												
.05	—	—	—	—	0–1	.33	0–0	0	—	—	—	—
.10	—	—	0–0	0	0–3	1.0	4–7	5.3	0–3	1.0	—	—
.15	0–0	0	0–1	.33	1–4	2.67	3–9	6.67	—	—	—	—
.20	0–0	0	0–2	1.0	4–5	3.67	8–11	9.33	0–11	6.0	—	—
.25	0–1	.33	1–2	1.67	—	—	—	—	—	—	—	—
.30	0–1	.67	—	—	5–6	5.67	—	—	0–11	6.0	—	—
.40	—	—	2–4	3.0	—	—	16–18	17.0	0–12	7.0	—	—
.50	1–2	1.67	—	—	—	—	—	—	—	—	0–0	0
.60	—	—	—	—	—	—	—	—	4–9	9.3	—	—
.70	—	—	—	—	—	—	—	—	—	—	0–0	0
.80	—	—	—	—	—	—	—	—	—	—	—	—
.90	—	—	—	—	—	—	—	—	—	—	0–1	0.3
1.00	—	—	—	—	—	—	—	—	—	—	0–2	.67
2.00	—	—	—	—	—	—	—	—	—	—	0–5	2.0

[a] Three replications.
[b] Figures underlined were obtained at twice the minimum effective dosage.

repellents was thus caused by a combination of greater initial effectiveness and better retention.

The initial and residual effectiveness of the repellents in relation to the length of the side chain is shown in figure 1.

The index of initial effectiveness of each repellent was obtained by dividing the minimum effective dosage of 1854 (the compound with the fewest carbons) by the minimum effective dosage of each repellent. The index of residual effectiveness was obtained by dividing the days that each repellent remained effective at twice its minimum effective dosage by the days 1854 remained effective at twice its minimum effective dosage.

AMIDES.—Tests were conducted in the same manner with the corresponding series of cyclohexane aliphatic amides. The results are presented in tables 3 and 4. Extending the length of the structural chain by the addition of 1 carbon increased the initial repellency, whereas the addition of 2 and 3 carbons resulted in a progressive decrease in repellency. The minimum effective dosage was 0.25 gram for cyclohexanecarboxamide (20458) and cyclo-

Table 3.—Number of bites per minute by *Aedes aegypti* through stockings treated with a series of cyclohexane aliphatic amides.[a]

Dosage (Grams)	20458		20837		20827		20816	
	Range	Average	Range	Average	Range	Average	Range	Average
0.0312	10+–10+	10+	10+–10+	10+	10+–10+	10+	10+–10+	10+
.0625	10+–10+	10+	0–10+	6.6	6–10+	8.6+	10+–10+	10+
.125	0–10+	6.3	0–3	1.3	0–2	1.3	10+–10+	10+
.25	0–0	0	0–0	0	0–4	2.3	4–10+	6.6+
.5	0–0	0	0–0	0	0–0	0	4–10+	7+

[a] Three replications.

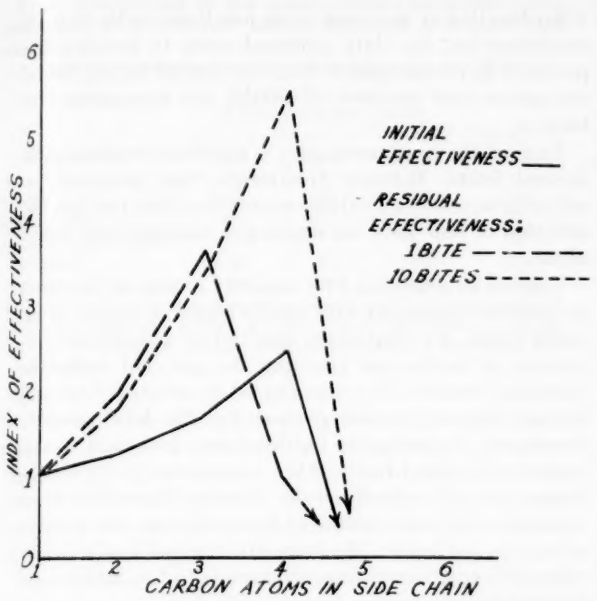

FIG. 1.—Relative effectiveness of cyclohexane aliphatic acids as repellents in relation to length of side chain.

Table 4.—Days stockings treated with a series of cyclohexane aliphatic amides remained effective against *Aedes aegypti*.[a]

Dosage (Gram)	20458 Range	20458 Average	20837 Range	20837 Average	20827 Range	20827 Average	20816 Range	20816 Average
Less than 1 bite								
0.0312	0–0	0	0–0	0	0–0	0	0–0	0
.0625	0–0	0	0–0	0	0–0	0	0–0	0
.125	0–0	0	0–0	0	0–0	0	0–0	0
.25	0–0	0	1–3	2.0	0–1	0.33	0–0	0
.5	1–1	1.0	2–4	3.0	0–2	1.3	0–0	0
Less than 10 bites								
.0312	0–0	0	0–0	0	0–0	0	0–0	0
.0625	0–0	0	0–0	0	0–0	0	0–0	0
.125	0–0	0	0–0	0	0–3	1.0	0–0	0
.25	0–0	0	2–4	2.3	3–8	6.0	0–0	0
.5	1–1	1.0	4–8	5.3	8–16	11.0	0–1	0.33

[a] Three replications.

hexaneacetamide (20837) and 0.5 gram for cyclohexanepropionamide (20827). However, at 0.125 gram the carboxamide allowed 6.3 bites, the acetamide 1.3. Cyclohexanebutyramide (20816) did not provide initial protection at any dosage. The residual repellency (less than 10 bites per minute) increased progressively with the addition of 1 and 2 carbons and then decreased.

Field and Laboratory Evaluations of Lead Arsenate, Wettable Sulfur and Hydrated Lime against the Plum Curculio[1]

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Prior to the introduction of the synthetic organic insecticides lead arsenate was the standard insecticide used for control of the plum curculio, *Conotrachelus nenuphar* (Hbst.). It is still extensively used in control of the species on cherry and apple. While it has been generally assumed that control was primarily the result of direct poisoning by ingested toxicant other factors were believed to be involved. Conceivably, control could occur as a result of one or more factors such as repellency causing the beetles to desert the tree, reduced feeding and oviposition occasioned by the presence of residue or death resulting directly from the toxic action of the insecticide. The influence of these factors in field control has not been conclusively determined, due chiefly to experimental difficulties. In field tests the fate of insects exposed to treatment can not be readily ascertained while laboratory studies introduce unnatural conditions which likely influence the behavior of the test insects.

The response of beetles to the spray operation and the presence of residue has been considered by several workers. Chapman (1938) conducted studies in which beetles were released in trees prior to spraying and recovered by jarring at intervals thereafter. The spray operation dislodged approximately 10% of the beetles. Thereafter beetles gradually deserted the trees but whether due to repellency or death was not concluded. Garman & Zappe (1929) reported that beetles deserted the trees within 24 hours after spraying but no dead beetles were recovered from sheets placed beneath the trees. It was concluded that possibly a fatal dose was obtained shortly after the trees were sprayed and the beetles went elsewhere to die.

The evidence gained by various workers from field and laboratory tests differs widely with respect to longevity of treated insects and ultimate cause of death. Bobb (1952) reports 33.4% mortality of beetles following 68 days exposure to treated fruit and foliage. Other investigators have reported complete kill in 4 days or more (Garman & Zappe 1929). Such discrepancy can be explained in part by differences in susceptibility of test insects, overwintering beetles being more susceptible than summer brood adults (Smith 1954). It seems generally agreed that the presence of lead arsenate residue results in a reduction in feeding and oviposition (Bobb 1952, Lieby & Gill, 1923, Snapp 1930, Smith 1954, Whitcomb 1929).

As lead arsenate is rarely applied alone consideration has been given to the influence on control of other components of spray mixtures. Lime sulfur alone has been reported (Chapman 1938) to account for control varying from 5 to 50% depending on severity of the infestation and it was reasoned by the writer that similar results would be expected with such materials as elemental sulfur and hydrated lime. Other workers (Garman & Zappe 1929, Snapp & Thompson 1932) reported lime sulfur, sulfur and hydrated lime to be ineffective.

The present report covers laboratory and field in-

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vestigations on response of beetles to treatment with combinations of lead arsenate, hydrated lime and wettable sulfur. The object of field studies was to obtain data under natural conditions which could be compared with laboratory findings. It is recognized that direct comparisons between these field and laboratory results are not entirely valid as the laboratory reared insects employed in these tests are not so readily killed as are the overwintering adults.

FIELD TESTS.—It was planned to compare the effectiveness of the various sprays on both cherry and prune. Unfortunately, the set of fruit in the prune orchard selected was sparse so that adequate numbers of trees bearing uniform loads could not be selected for counts. While the data are unsatisfactory for critical comparison between various treatments they are included to show the contrast in results obtained under different experimental conditions. In addition to control data, records were taken from cherry on the number of beetles inhabiting trees following treatments. This was accomplished by jarring the beetles from trees at intervals of 5 days following the first two sprays. The captured beetles were counted and released beneath the host trees. Collections were made from approximately 14 trees in each treatment, individual trees being jarred only once. Similar collections were not made from prune because the jarring operation dislodges prune fruits as they increase in size.

Control on Cherry.—Plots were established in a 10-year-old Montmorency orchard and consisted of individual rows of 12 trees which were replicated three times. Sprays were applied with a standard hydraulic rig. Three sprays were applied at approximately 10-day intervals beginning on May 26, 1954, as fruits reached the "shuck-split" stage. The following materials and combinations were employed per 100 gallons of water:

a. wettable sulfur, 5 lbs.
b. wettable sulfur, 5 lbs.; hydrated lime, 2 lbs.
c. wettable sulfur, 5 lbs.; hydrated lime, 2 lbs.; lead arsenate, 2 lbs.

Results were determined by collecting all injured fruits from selected trees during the course of the season. Records were taken from trees selected for uniformity of size and crop as well as location with respect to hibernation quarters of the beetles. Infested fruits were picked from the trees and held in an insectary where the mature larvae were counted as they emerged. The picking of infested fruits was necessary as infested cherry fruits do not drop as do peaches and prunes. The per cent larvae maturing when handled in this manner is less than under natural conditions but the data obtained serve to indicate the period of larval emergence. Records on fruit injury, larval emergence and recovery of beetles are summarized in table 1.

Each of the treatments gave a significant reduction in injured fruits. Between treatments, lead arsenate resulted in increased effectiveness over the other two but the addition of lime gave no significant increase over sulfur alone.

Control as determined by recovery of mature larvae is in essential agreement with results based on counts of injured fruits. All treatments resulted in a significant reduction in beetles per tree and the per cent reduction coincided closely with control based on counts of fruit and larvae. Agreement was poorest for the lead arsenate treatment, reduction in beetles being less than actual control of injured fruits. This is apparently due to the lower rate of oviposition by beetles inhabiting trees sprayed with lead arsenate as surmised from the number of injuries per beetle. The oviposition rate of beetles in the other two treatments was comparable to the rate for untreated insects.

Control on prune.—A 10-year-old Fellenberg prune orchard was selected for this test and treatments made in the manner described for cherry. Results were determined by the recovery of mature larvae from dropped fruits. The results of these tests are shown in table 2 and are shown graphically with comparable data from cherry in fig. 1. As suggested earlier these data are influenced by the sparse set of fruit in the test orchard and are insufficient for critical comparison. It is apparent, however, that in all cases control was less on prune than on cherry but the reasons for this difference are not apparent. Such difference is in keeping with grower experience over the years, that adequate control of the plum curculio with lead arsenate is obtained on cherry while control on prunes is unsatisfactory. It is of interest to note that the curves representing larval emergence from untreated trees of the two hosts differ in slope, the difference being greater than could reasonably be accounted for by differences in seasonal development of the two host fruits.

In the foregoing it has been shown that sulfur alone accounts for considerable control of the plum curculio, 71% in the case of cherry. The addition of lime caused little if any increase in control on cherry although the fragmentary data from prune suggested increased control. Lead arsenate accounted for an increase in control in

Table 1.—Summary of injured fruits, mature larvae and beetles recovered per tree following spray treatments.[a]

Treatment	Injured Fruits per Tree			Per Cent Control	Mature Larvae per Tree	Per Cent Control	Aver. Beetles per Tree	Per Cent Reduction	Injured Fruits per Beetle
	Min.	Max.	Aver.						
Sulfur	41	356	170	71	42	78	2.1	72	81
Sulfur+lime	30	262	146	75	42	78	1.6	79	91
Sulfur+lime+lead arsenate	4	34	17	97	2	99	0.7	91	24
Untreated	336	1055	591	—	192	—	7.5	—	79
L.S.D. at 5% level			103				2.7		

[a] Sprays applied May 26, June 4, June 15, 1954. The following quantities were used per 100 gals.: wettable sulfur, 5 lbs.; hydrated lime, 2 lbs.; lead arsenate, 2 lbs.

Table 2.—Control of the plum curculio on prune using combinations of wettable sulfur, hydrated lime and lead arsenate.

Treatment[a]	Number of Larvae Recovered per Tree			Per Cent Control
	Min.	Max.	Aver.	
Sulfur	19	323	125	38
Sulfur+lime	36	188	99	51
Sulfur+lime+lead arsenate	8	55	32	84
Untreated	124	296	203	

[a] Sprays applied May 27, June 5, June 15, 1954. The following quantities were used per 100 gals.: wettable sulfur, 5 lbs.; hydrated lime, 2 lbs.; lead arsenate, 2 lbs.

both cases. Control for all treatments was higher on cherry than on prune, an observation supported by grower experience and one which can not be explained at this time. The larval emergence curves for untreated prune and cherry trees differed, suggesting that the rate of larval development is influenced by the host fruit.

The reduction in injury by treatments with lead arsenate is apparently due in a large measure to repellency and reduced oviposition among the females remaining in the trees. A 91% reduction in number of beetles resulted from the combination including lead arsenate while a 97% reduction in injured fruits occurred. In the other two treatments reduction in beetles and in injury coincided closely suggesting that repellency rather than reduced oviposition was chiefly responsible for control. Wetting of the insects with spray at the time of treatment apparently has no ill effect based on response of laboratory insects which were immersed in the various spray mixtures.

Laboratory Tests.—In the laboratory tests females were confined on treated fruits to determine the effect of treatment on rate of feeding, oviposition and mortality. The test insects were laboratory reared females approximately 4 weeks old and near the peak of oviposition activity. Rearing and testing were conducted at a temperature of 80° F. Insects were confined individually under jelly glasses supported on a screen rack, using 25 females per treatment. Green thinning apples approximately 1 inch in diameter were used for food. Fruits were treated by spraying on a turntable to the point of run-off using a DeVilbiss paint sprayer operated at 20 lbs. pressure. Materials were used at the same concentration employed in field tests. New apples were provided each day and records maintained on the number of feeding punctures and eggs produced each day by the individuals. Fruits were inspected with the aid of a binocular microscope.

In determining results of these tests two factors are of primary interest, the effect of treatment on rate of mortality and its effect on feeding and oviposition.

Mortality.—The time-mortality curves determined for the test insects are shown in fig. 2. In addition, the curve plotted for insects deprived of food and water is also shown. The highest rate of mortality among treatments resulted from the combination of the three materials followed by sulfur alone. The sulfur treatment was characterized by high initial rate of mortality followed by a flattening of the curve. Lime proved least effective but the combination of lime and lead arsenate was more effective than lead arsenate alone. Complete mortality occurred after 46 days in the case of the combination of the three materials, longer periods being required for the other treatments. It is interesting to note that with lead arsenate alone 50% mortality was reached in 19 days and complete mortality only after 51 days. This bears out the observation of other workers that the material is not highly toxic and some individual curculios show surprisingly little susceptibility to lead arsenate residue.

The evidence presented here particularly in the case of the combined materials raises the question as to whether death is due to poisoning or starvation. The time-mortality curve for insects deprived of food and water coincides closely with the curve for the combination of materials. Furthermore, 44% of these test insects died without feeding while not over 4% in any other treatment

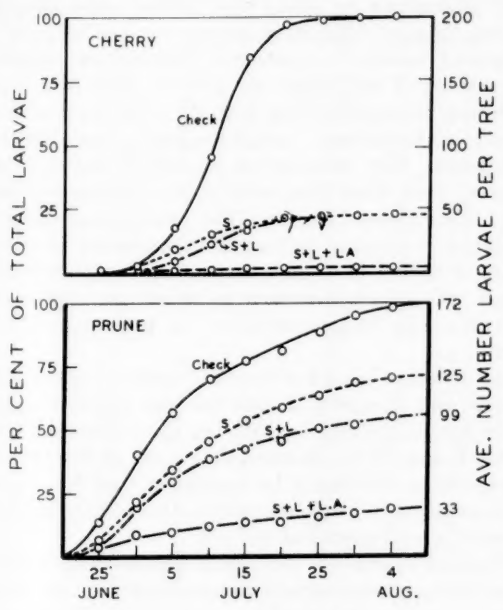

Fig. 1.—The emergence of plum curculio larvae from prune and cherry fruits treated with combinations of lead arsenate (LA), lime (L) and sulfur (S).

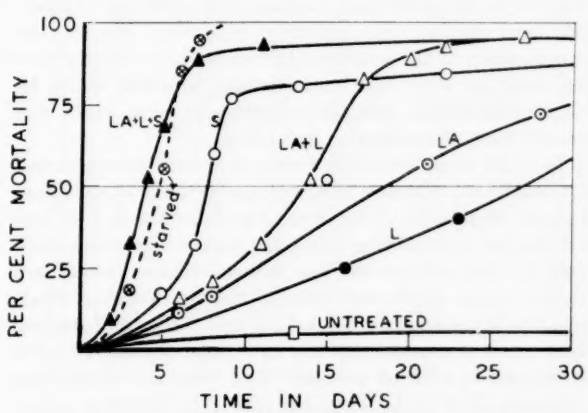

Fig. 2.—Time-mortality curves for plum curculio beetles feeding on apples treated with lead arsenate (LA), lime (L) and sulfur (S).

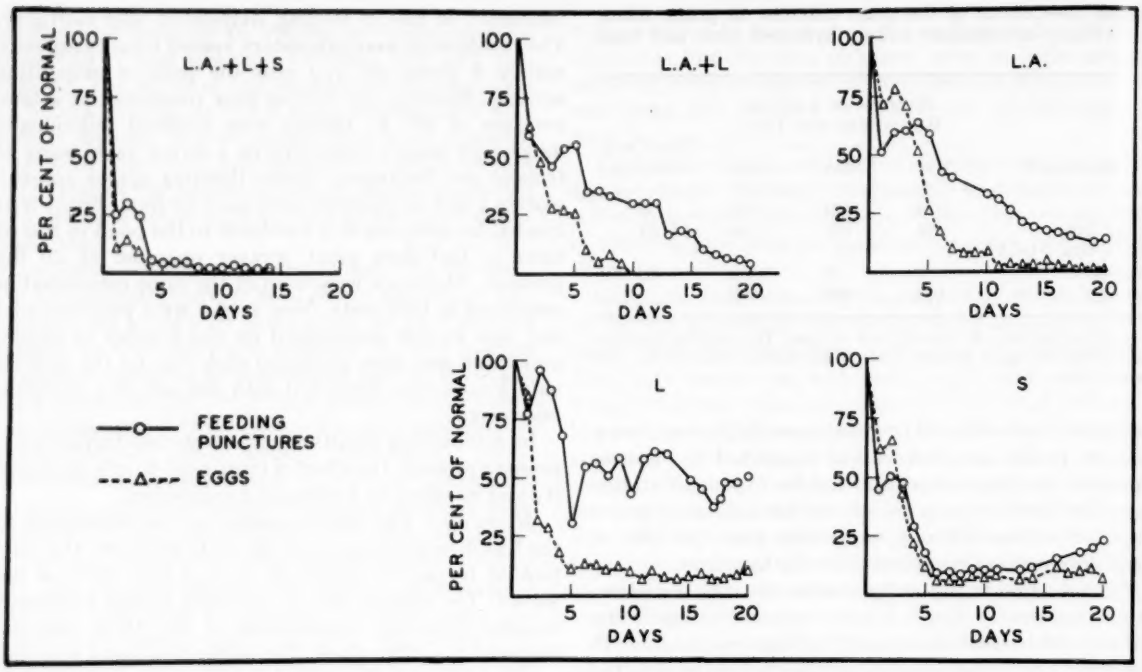

FIG. 3.—The per cent reduction in injury due to death and subnormal feeding of 25 female curculio fed on apples treated with lead arsenate (LA), lime (L) and sulfur (S).

died without feeding. This evidence and the similarity with the time-mortality curve suggest that death is due chiefly to starvation. Although the curve for the sulfur treatment resembles the starvation curve in slope, death is not believed due to this cause as the individuals fed extensively as will be discussed later.

Feeding and oviposition.—The gross effect of treatment on rate of feeding and oviposition reflects reduction due to both death and subnormal feeding of survivors. The average daily rate of activity prior to treatment was 14 feeding punctures and 5 eggs per female or 350 feeding punctures and 130 eggs for each group of test insects. In considering the degree of reduction caused by treatments, the group figures given above were used as a base for calculating the per cent of normal activity for the various groups. These data are shown in fig. 3. All treatments resulted in an immediate reduction in injury by oviposition and feeding. The greatest reduction was for the combination of three materials, followed by sulfur alone; lead arsenate plus lime; lead arsenate; and lime alone. In all treatments the relative reduction in oviposition was greater than the reduction in feeding.

In order to differentiate between reduction occasioned by death and that due to subnormal activity of survivors the rate of activity of survivors was determined. This was arrived at by dividing the total injury occurring each day by the number of live insects. These results are shown in fig. 4. No survivors of the combination treatment produced eggs after 4 days and the rate of feeding continued at a uniformly low rate until death. With the combination of lead arsenate and lime no oviposition occurred after 10 days and the rate of feeding was higher than where sulfur was added. The results with lead arsenate alone did not differ greatly from the combination of lead arsenate and lime although oviposition did not cease entirely with lead arsenate alone. In the case of sulfur alone, activity tended to increase following the low point reached after 6 days.

It is noteworthy too that there appears to be a critical level of feeding activity below which oviposition does not occur. This level appears to be approximately 5 feeding punctures per day or 35% of normal feeding.

In attempting to study the factors accounting for control, interest ultimately centers on the response of individual insects to treatment. Records on longevity and activity of individuals are given in table 3, arranged according to total feeding activity. The most striking feature is the extreme variations among individuals of each group. The combination treatment was strikingly different from other treatments in the number of insects which died without feeding. The extremes among individuals were greatest in the sulfur treatment, those surviving a period of 7 days continuing for an extended period. With such extremes as shown in table 3 it is difficult to cite "typical behavior" for individuals of each group.

For instance, No. 18 of the combination treatment fed heavily and deposited 5 eggs the day before its death while No. 21 did not feed during the 3 days preceding death. It can not be reasoned that death of No. 18 is due to starvation nor can it be concluded that No. 21 died of poisoning rather than starvation. Quite likely mortality is due to a combination of the two.

While all treatments containing lead arsenate would be expected to possess certain common features, sulfur and lime, generally considered non-toxic, might be expected to differ widely. Starvation apparently accounts for considerable mortality in the case of the sulfur treatment,

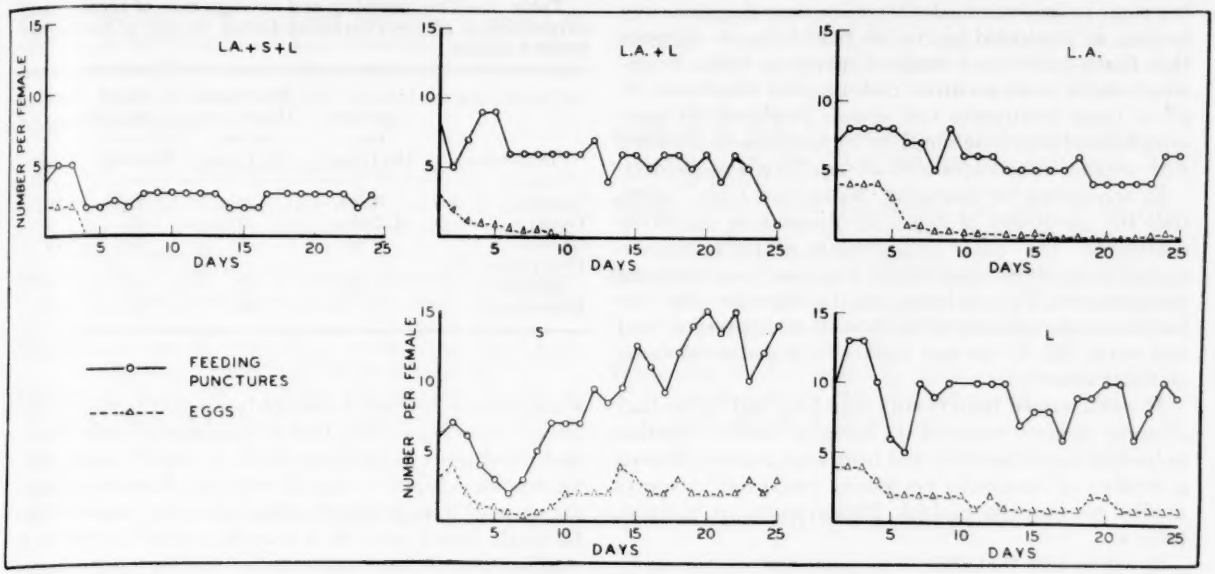

FIG. 4.—The daily rate of feeding and oviposition by surviving female curculio fed on apples treated with lead arsenate (LA), lime (L) and sulfur (S).

Table 3.—Record of longevity, total feeding and oviposition by individual plum curculios fed on treated apples.

	TREATMENT[a]														
	Lead Arsenate Lime Sulfur			Lead Arsenate Lime —			Lead Arsenate — —			— Lime —			— — Sulfur		
INSECT No.	Feeding	Eggs	Days Survival	Feeding	Eggs	Days Survival	Feeding	Eggs	Days Survival	Feeding	Eggs	Days Survival	Feeding	Eggs	Days Survival
1	0	0	2	0	0	2	20	9	5	0	0	1	0	0	1
2	0	0	4	4	0	3	21	7	5	2	2	3	6	2	7
3	0	0	3	23	9	3	36	2	5	36	7	7	8	3	3
4	0	0	2	36	10	7	46	20	8	39	8	4	9	0	5
5	0	0	1	39	9	5	48	16	7	44	15	5	13	8	3
6	0	0	3	52	8	10	52	7	10	130	50	22	16	4	7
7	0	0	2	54	3	16	72	19	11	134	3	22	17	8	5
8	0	0	2	57	12	8	76	22	15	139	72	44	18	6	6
9	0	0	2	58	6	12	86	9	11	144	6	41	20	12	6
10	0	0	2	68	0	16	119	31	10	146	44	26	22	6	6
11	0	0	6	69	14	15	122	24	31	151	4	26	29	16	6
12	1	0	3	87	17	9	126	31	22	157	30	17	31	20	6
13	2	1	2	92	8	14	130	22	13	178	31	33	34	9	6
14	6	0	3	92	12	13	140	28	21	185	20	30	36	15	5
15	7	4	4	92	20	15	148	18	11	193	59	41	41	13	7
16	15	3	6	93	29	11	149	30	11	215	41	21	41	19	6
17	21	14	6	98	2	19	149	39	26	236	50	24	43	8	6
18	23	12	3	105	29	12	164	14	37	237	49	15	44	23	5
19	25	9	4	114	6	14	171	1	33	311	49	36	57	15	7
20	26	2	4	118	24	16	172	17	37	323	102	61	60	17	12
21	31	7	6	119	0	11	176	22	20	403	118	39	153	11	21
22	35	17	5	154	23	15	177	61	30	419	106	33	270	14	31
23	73	7	11	160	4	21	185	0	51	421	67	30	398	61	34
24	144	24	37	200	23	26	207	37	27	430	93	42	419	147	68
25	243	27	46	299	30	53	260	23	37	586	50	97	1125	372	99
Average	26	5	7	91	12	14	122	20	20	210	43	29	116	32	15

[a] Untreated insects averaged 14 feeding punctures and 5 eggs per day. L.S.D. at 5% level: Feeding—75.7. Eggs—21.6. Survival—9.1.

but some individuals died after a few days despite heavy feeding, as illustrated by No. 18. Such behavior suggests that death occurs as a result of ingesting sulfur. Somewhat similar cases occurred with the lime treatment. In all of these treatments test insects displayed no toxic symptoms of poisoning similar to those of insects poisoned with organic insecticides such as parathion and dieldrin.

In accounting for mortality due to "non-toxic" materials the possibility of death by desiccation should be considered. The water requirements of plum curculio appear to be much more critical than food or nutritional requirements. This is borne out by the fact that the beetles can survive only a few days when deprived of food and water (fig. 2), but can survive for a matter of weeks on water alone.

It was assumed from results with lime and sulfur that all spray residues would likely induce a similar reduction in feeding and oviposition and tests were conducted using a number of fungicides commonly employed in sprays applied for curculio control. These results are given in table 4.

Table 4.—The mortality and average rate of feeding and oviposition of 20 plum curculios during 10 days of feeding on treated apples.

Treatment	Amount per 100 Gals.	Per Cent Mortality After 10 Days	Daily Rate per Female	
			Feeding	Eggs
Captan	2 lbs.	0	19	10
Fermate	1.5 lbs.	0	16	9
Glyodin	1 qt.	0	22	10
Phenylmercuric acetate	1 pt.	0	18	9
Untreated	—	0	17	11

It will be seen that little or no reduction was caused by these materials. The fermate particularly would have been expected to cause reduced activity because of its heavy, conspicuous residue. An obvious chemical difference between these materials is noted, the latter being organic in nature.

Discussion.—A considerable body of evidence from field and laboratory tests has been presented which establishes several pertinent facts relative to plum curculio control. These are summarized below:

1. All field treatments involving lead arsenate, lime and sulfur resulted in marked reduction in injury, greater reduction occurring on cherry than on prune.
2. The extent of injury was roughly proportionate to the number of beetles recovered from trees following treatment.
3. While all treatments caused some mortality in laboratory tests, the time-mortality curves indicate that mortality alone does not account for the reduction of beetles in treated trees.
4. Residues of each of the materials caused reduced feeding and oviposition in laboratory tests.
5. Oviposition under laboratory conditions was proportionate to feeding activity.
6. Longevity was reduced when the test insects were fed on fruits treated with these materials alone or in combination.

In attempting to reconcile these findings with control under practical conditions two considerations are paramount; first, in practice the fate of insects exposed to treatment is not known and second, insects confined on treated fruits are confronted with a "feed or die" situation which does not occur in the orchard. Despite these experimental considerations the data presented provide a basis for speculating on the manner in which control is obtained under field conditions. Accordingly the following concept is proposed: Residues of these materials are distasteful to the beetles, causing them to desert the trees in search of more favorable situations. This results in dispersal over the area and probably a concentration of beetles in adjoining untreated trees. Where more favorable situations are not found some of the insects remain in the treated trees where they feed at subnormal levels. Under such conditions oviposition which is chiefly responsible for economic injury is sharply reduced. Continued feeding on residue, particularly of lead arsenate, leads in time to death. Based on such a concept, control is due to a combination of dispersal, reduced feeding and oviposition, and finally death.

A number of variables would logically influence the degree of control accounted for by these interacting factors. Chief among these is probably population density. As the plum curculio has been shown to avoid overcrowding even in the absence of control measures (Chapman 1938) it is conceivable that in dense populations the number of beetles remaining in treated trees would approach the "saturation" point in which case little or no control would be demonstrated. This factor may account for the ineffectiveness of lead arsenate treatments as cited by Crandall (1905). On the other hand, in light infestations a high degree of control would be expected. In the cherry orchard employed in these tests a rather heavy population occurred but the adjoining area provided suitable alternate host trees both wild and cultivated. It has been established that the effectiveness of lead arsenate is also influenced by such factors as the presence of moisture on the sprayed surface (Smith 1954).

Summary.—Field and laboratory tests were conducted to determine the effect of lead arsenate, hydrated lime and wettable sulfur on the control of the plum curculio. In field tests all treatments effected some control, the same materials giving higher control on cherry than on prune. The degree of control coincided roughly with the number of beetles inhabiting the trees following treatment. In laboratory tests where individual females were confined on treated fruits, a reduction occurred in rate of feeding, oviposition, and longevity. In all treatments a positive correlation between feeding and oviposition occurred. The death of some individuals was presumably due to desiccation rather than toxicity of ingested spray deposit as evidenced by failure of these individuals to feed prior to death. Insects confined on fruits treated with lead arsenate reached 50% mortality after 19 days and complete mortality after 51 days indicating that the inherent toxicity of lead arsenate is low.

In reconciling these findings with control under practical conditions the following concept is proposed: residues of these materials are distasteful to the plum

curculio causing them to desert the trees in search of more favorable situations. Those remaining in the treated trees feed at sub-normal levels resulting in reduced oviposition while continued feeding ultimately accounts for death of the beetles.

REFERENCES CITED

Bobb, M. L. 1952. The life history and control of the plum curculio in Virginia. Virginia Agric. Expt. Sta. Bull. 453. 30 pp., illus.

Chapman, P. J. 1938. The plum curculio as an apple pest. New York State Agric. Expt. Sta. Bull. 684. 75 pp., illus.

Crandall, Charles S. 1905. The curculio and the apple. Illinois Agric. Expt. Sta. Bull. 98. 93 pp., illus.

Garman, Philip, and M. P. Zappe. 1929. Control studies on the plum curculio in Connecticut apple orchards. Connecticut Agric. Expt. Sta. Bull. 301. 54 pp., illus.

Leiby, R. W., and John B. Gill. 1923. The plum curculio on peaches in North Carolina—Its life history and control. Bull (no number) North Carolina Dept. Agric. 23 pp., illus.

Smith, E. H. 1954. Factors influencing the susceptibility of the plum curculio to lead arsenate. Jour. Econ. Ent. 47(5): 871–9.

Snapp, Oliver I. 1930. Life history and habits of the plum curculio in the Georgia peach belt. U. S. Dept. Agric. Tech. Bull. 188. 90 pp., illus.

Snapp, Oliver I., and J. R. Thompson. 1932. A progress report on feeding tests and spraying and dusting experiments for the control of the plum curculio. Jour. Econ. Ent. 25: 811–15.

Whitcomb, W. D. 1929. The plum curculio in apples in Massachusetts. Massachusetts Agric. Expt. Sta. Bull. 249. 52 pp., illus.

Control of the Carrot Weevil Attacking Parsley[1]

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The carrot weevil, *Listronotus oregonensis* (Lec.), has been a species of economic importance to growers of parsley on Long Island for a number of years. The recommendations of Boyce (1927) are difficult to follow under the present conditions of urbanization and the consequent reduction of farm land especially in the more western area of Long Island. Currently, it is not always possible to rotate host with non-host crops as suggested in the early report. However, Chupp & Leiby (1953), in a later recommendation for New York growers, have included rotation as a means of control although they do suggest that a 5% DDT or a 1% parathion dust may be of value.

Hill (1948) considered a 3% DDT dust ineffective although he submitted that the lack of efficiency may have been one of improper timing. DDT and BHC were found to be effective as foliar sprays by Bare (1950) and Wright (1953) but methoxychlor, chlordane and toxaphene gave poor control. In addition, the latter found that parathion and dieldrin sprays were fairly effective against the weevil and similar applications of aldrin and heptachlor inferior. Apple pomace-calcium arsenate baits also gave poor results. The inferiority of the apple pomace bait counters the proposal of Pepper (1942) that such baits are the most effective means of weevil control.

The object of the experiment in 1956 was to test various organic compounds currently available to determine whether the carrot weevil could be controlled in parsley by means other than rotation of crops.

METHODS AND MATERIALS.—An experimental block was established on the farm of a cooperating grower at Farmingdale, Long Island. In past seasons, the carrot weevil was responsible for severe damage to parsley and hymicha (Hamburg parsley) grown on this particular farm and it was evident from a preplanting survey that a large number of overwintered weevil adults was present, especially in one location where a planting of parsley had been made in 1955. This area was situated approximately 50 feet east of the experimental block.

The block was divided into plots six rows wide and 36.3 feet long (0.01 acre). Four plots, replicated four times in a randomized block design, received pre-emergence applications. Postemergence treatments were made to six others. The materials used in the pre-emergence treatments were as follows: heptachlor 25% and dieldrin 10% granulated formulations; heptachlor, 2-pound- and dieldrin, 1.5-pound-per-gallon emulsifiable concentrates. The granulated insecticides were broadcast with a rotary hand duster. Spray emulsions were distributed as uniformly as possible over the plots by means of a 2-gallon watering can having a finely perforated spout. The emulsions were applied at the rate of 100 gallons per acre. It was planned to apply the granulated materials at a rate of 4 pounds of actual toxicant per acre but the output of the apparatus used to disperse the formulations could not be controlled accurately and, as a result, somewhat higher doses were delivered. Pre-emergence treatments were applied as blanket soil-surface applications, without subsequent tillage, on April 24, 10 days after seeding and 13 days prior to initial emergence.

Postemergence treatments were begun on May 16 after the parsley had reached a height of approximately ½ inch. Applications of the water emulsions were made by means of a hand-operated knapsack sprayer. The plants and the surrounding soil were thoroughly sprayed at the rate of 100 gallons per acre. The following emulsifiable concentrates were used: toxaphene, 6 pounds actual toxicant per gallon; chlordane, 8 pounds actual; dieldrin, 1.5 pounds actual; heptachlor, 2 pounds actual. The dosages of actual toxicant applied are presented in table 1.

Granulated formulations of heptachlor and dieldrin were included in the postemergence treatments and were dispensed as previously described. The quantity of actual toxicant used varied with each application. At the first postemergence treatment-date, dieldrin and heptachlor were applied at rates of 4.64 and 4.58 pounds of actual toxicant per acre, respectively. At the time of the second

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Table 1.—Carrot weevil injury to parsley after insecticidal treatment. 1956.

Treatment	Pounds Actual Toxicant per Acre	Number Injured Plants[a]	Per Cent Reduction
Pre-emergence			
Heptachlor, 25%, gran.	5.15	3.8	94.3
Dieldrin, 10%, gran.	4.49	7.0	89.4
Heptachlor, E	4.0	24.5	62.7
Dieldrin, E	4.0	15.3	76.8
Postemergence			
Toxaphene, E	3.0	23.5	64.3
Chlordane, E	1.0	23.3	64.6
Dieldrin, E	0.375	3.3	95.1
Heptachlor, E	1.0	3.0	95.4
Dieldrin, 10%, gran.	—[b]	2.5	96.2
Heptachlor, 25%, gran.	—[b]	0.5	99.2
Untreated check		65.8[c]	
L.S.D. at 1% level		13.02	
at 5% level		9.64	

[a] Sum of two counts and average of four replications.
[b] Consult section on "Methods and Materials" for respective dosages.
[c] Not included in analysis of variance.

application (June 7), 5.45 and 5.6 pounds actual were applied, respectively. The final postemergence application was split because of a difference in time of harvesting of the four replicates. The earlier harvested replicates were treated on July 13 whereas treatment of the remaining two was delayed until July 25. Dieldrin, 4.47 and 4.7 pounds, and heptachlor, 6.38 and 5.05 pounds actual, were applied at these dates, respectively.

Initially, applications were made to each of the plots in its entirety, however, due to the variation in market demand for the parsley crop, harvesting was done piecemeal by row, and it was possible to treat only the two inner rows (from which the data were taken) of the plots on July 13 and 25 soon after they had been harvested.

The severity of the carrot weevil infestation, and the effectiveness of the insecticidal treatments, were determined by counting the number of injured plants in the center 20 feet of each of the inner two rows of each plot. The injured plants could be recognized from the symptoms shown, i.e., chlorosis and wilting due to the tunneling of the larvae. These plants were removed upon counting so as not to complicate subsequent examinations. The first count was made on June 29. A second count was made on August 15 in the replicates receiving the earliest final treatment and the other replicates were examined on August 27. Thus, final counts were made 33 days after final treatment. A summary of these data is presented in table 1.

RESULTS AND DISCUSSION.—Although all of the treatments led to a significant reduction in carrot weevil injury, postemergence treatments of toxaphene and chlordane were significantly inferior to all other treatments with the exception of a pre-emergence treatment of granulated heptachlor. This finding is in complete agreement with the results reported by Bare (1950) and Wright (1953). Postemergence treatments of heptachlor and dieldrin, in emulsifiable or granulated form, gave excellent control. It should be noted that much higher doses of the latter type formulations were used. These data suggest that an insecticide-fertilizer mixture containing either dieldrin or heptachlor may offer an excellent means of controlling the carrot weevil when applied as a broadcast treatment to parsley. It is not possible to surmise from the data obtained whether the granulated formulations would be as effective if applied at dosage levels comparable to those of the emulsifiable formulations. In contrast to the report of Wright (1953), heptachor, in emulsifiable form, proved to be very effective for carrot weevil control at a practical rate of application.

A single pre-emergence application of granulated dieldrin or heptachlor also gave good control but it may be noted that the trend was in the direction of decreased effectiveness when compared with postemergence applications of considerably less emulsifiable insecticide.

In verification of the terrestrial migratory habit of the alate carrot weevil adult, a significant decrease in infestation was evidenced across the breadth of the experimental block from the first to the fourth replicates. The untreated plots in the replicates farthest from the weevil-infested area to the east of the planting averaged 51 infested plants compared to 80.5 plants injured in the more proximal untreated plots. These results suggest that the application of higher doses of toxicant to sections of a planting most vulnerable to attack may control not only the weevils therein, but may effectively prevent the movement of adults to the more removed sections as well.

SUMMARY.—Emulsion sprays and broadcast treatments of dieldrin and heptachlor, at practical levels, gave excellent control of the carrot weevil in parsley when application was made to newly emerged plants and repeated after each cutting. Treatment appeared to be effective for a minimum of 22 to 33 days. Pre-emergence treatments of these insecticides in granulated form was suggested as a possible means of control. The reported results and those of other workers indicate the decided inferiority of toxaphene and chlordane for control of the carrot weevil.

REFERENCES CITED

Bare, O. S. 1950. Insect control experiments. *In* Nebraska Agric. Expt. Sta. Ann. Rept. **63**: 85.

Boyce, A. M. 1927. A study of the biology of the parsley stalk weevil *Listronotus latiusculus* Boheman. Coleoptera: Curculionidae. Jour. Econ. Ent. **20**(6): 814–21.

Chupp, C., and R. W. Leiby. 1953. The control of diseases and insects affecting vegetable crops. New York (Cornell) Agric. Expt. Sta. Ext. Bull. **206**: 39.

Hill, R. E. 1948. Insect control experiments. *In* Nebraska Agric. Expt. Sta. Ann. Rept. **61**: 56.

Pepper, B. B. 1942. The carrot weevil, *Listronotus latiusculus* (Bohe), in New Jersey and its control. New Jersey Agric. Expt. Sta. Bull. **693**: 1–20.

Wright, J. M. 1953. An investigation into the biology and control of the carrot weevil, *Listronotus oregonensis* (LeConte). Thesis for Degree of Ph.D., Univ. of Ill. (Unpublished)

Further Evidence for Autosomal, Multiple-Factor Inheritance of Chlordane Resistance in the German Cockroach[1]

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Grayson *et al.* (1956) have reported that the factors for chlordane resistance in the German cockroach, *Blattella germanica* (L.,) are autosomal; that apparently little or no dominance is involved in the activity of these factors; and that no evidence was obtained to indicate the influence of any cytoplasmic or sex-linked factors. These conclusions were based upon the results obtained from determining the orders of resistance of: (a) chlordane-resistant and non-resistant strains, (b) the F_1 progeny from reciprocal crosses, and (c) the progeny of selected back crosses.

The results reported in this paper show the relative toxicity of chlordane to: (a) the F_2 progeny of reciprocal crosses between chlordane-resistant and non-resistant strains of the German cockroach, (b) the progeny of pair matings within strains, and (c) cockroaches in successive generations of a chlordane-resistant strain in which selection was discontinued.

Procedure.—The cockroaches used in the mass matings were reared as previously described (Grayson 1951, Grayson *et al.* 1956). In the pair mating studies, however, the cockroaches were reared in 1-quart, glass jars, and kept at all times in a constant temperature cabinet at 30° C. and 55% relative humidity. Each adult pair was transferred to a new jar when the egg case hatched.

Three crosses of cockroaches were made for the pair mating studies. One consisted of males and females from a chlordane-resistant strain; a second was composed of males and females from a non-resistant strain; and the third cross consisted of mating females from a chlordane-resistant strain with males from a non-resistant strain (designated as X strain). Virgin females were used in making all of the pair matings. These were obtained by separating large nymphs from stock cultures, and observing them twice each day for removal of adults and separation of sexes.

The procedures in preparing formulations, treating the cockroaches, and taking mortality counts, were the same as previously described (Grayson *et al.* 1956), except that in the pair mating tests only about eight adult cockroaches were available for each sample. Most of the dosage-mortality data obtained were plotted on logarithmic-probability paper, and regression lines were fitted to the points by the method of least squares (Bliss 1935). These methods were not feasible for the data obtained from the treatment of progeny of pair matings, however, so a rough "index of resistance" was calculated for the male and female progeny of each parental pair in all strains and crosses. The index figure represents the sum of the products obtained by multiplying the reciprocal of each concentration with the corresponding mortality, a low index indicating greater resistance than a high one. Correlation coefficients were determined for the males and females of each strain (Snedecor 1946).

Results.—The regression lines indicating the toxicity of chlordane to F_2 progeny arising from reciprocal crosses between resistant and non-resistant strains are shown in figure 1. Although similar data have been

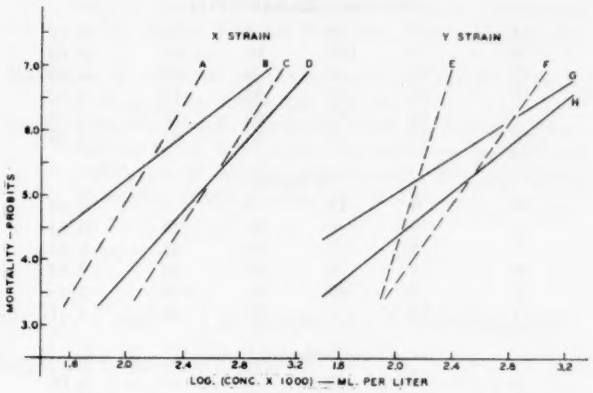

Fig. 1.—Mortality in F_1 and F_2 progeny reciprocal crosses after 10 days following treatment with chlordane. F_1, X-strain males, (A); F_2, X-strain males, (B); F_1, X-strain females, (C); F_2, X-strain females, (D); F_1, Y-strain males, (E); F_1, Y-strain females, (F); F_2, Y-strain males, (G); and F_2, Y-strain females, (H). The X strain resulted from crossing resistant females with non-resistant males, and the Y strain from non-resistant females and resistant males.

previously published (Grayson *et al.* 1956) for the parental strains and the F_1 progeny, the latter is included in dotted lines for comparative purposes. The X strain was produced by mating chlordane-resistant females and non-resistant males, whereas the Y strain orginated from crossing non-resistant females and chlordane-resistant males.

Data were obtained on the toxicity of chlordane to the progeny of from 20 to 25 pairs in each strain included in the pair mating studies. As these data were rather extensive, only representative results with female progeny are being presented (table 1). The correlation coefficient between the indices for male and female progeny of the same pairs was positive and significant for each strain.

The regression lines indicating the toxicity of chlordane to different generations of a strain formed from the chlordane-resistant strain, but reared through successive generations without further exposure to an insecticide, are shown in figure 2. Only the mortality data for females are shown, as those for the males were similar and would have necessitated another figure if they had been included.

The "b" values, indicating slopes of the regression lines, which were obtained when the dosage-mortality data in figure 1 were plotted on log-probit paper were as follows: For X strain—F_1 males, 3.7; F_2 males, 1.6; F_1 females, 3.3; F_2 females, 2.3; and for Y strain—F_1 males, 6.9; F_2 males, 1.4; F_1 females, 3.0; F_2 females, 1.7. Similar

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Table 1.—Toxicity of chlordane to female progeny of pair matings within strains and crosses.

Pair Number	Per Cent Mortality 10 Days Following Treatment at 4 Concentrations[a]				Index of Resistance[b]
	Chlordane-Resistant Strain				
14	0	0	0	22	0.01
6	0	100	12	67	0.26
17	20	0	0	25	0.68
10	29	0	33	17	1.02
2	33	87	67	78	1.42
16	67	77	33	91	2.49
	X Strain				
10	0	17	0	0	0.42
1	0	0	20	29	0.81
7	8	0	20	37	1.25
13	8	0	91	36	2.65
4	0	62	30	100	3.58
11	64	0	67	60	4.76
	Non-Resistant Strain				
8	0	40	0	12	8.00
23	0	0	0	100	33.33
9	0	43	33	89	89.16
3	0	71	33	100	120.83
13	11	100	50	100	174.04
6	40	100	75	100	227.97

[a] The concentrations (ml. per liter) were 3.0, 5.0, 7.0, and 15.0 for the chlordane-resistant strain; 0.25, 0.4, 0.5, and 0.7 for the cross; and 0.007, 0.01, 0.02, and 0.03 for the non-resistant strain.
[b] See text.

values for the dosage-mortality data in figure 2 were: resistant strain (P_1), 2.5; F_5 strain, 1.2; F_7 strain, 1.3; F_{12} strain, 1.4; F_{14} strain, 1.2; and non-resistant strain, 2.4.

Discussion.—As the simplest hypothesis for autosomal transmission of resistance we might consider that only a single locus is involved and that the gene for resistance and its allele for susceptibility are homozygous in the chlordane-resistant strain and the non-resistant strain, respectively. If so, the F_1 males and females of the X- and Y-strain crosses would be heterozygous and genetically identical in the two strains. Since the F_1 cockroaches are assumed to be heterozygous, the F_2 progeny would consist of varying genotypes and therefore would exhibit a wider variance of resistance to the insecticide. This was reflected in F_2 regression lines of greater slope in the X- and Y-strain progeny as compared with those of the F_1 progeny. There was no significant difference found between the males and females, respectively, of the two strains. The 95% fiducial limits indicated the populations to be similar.

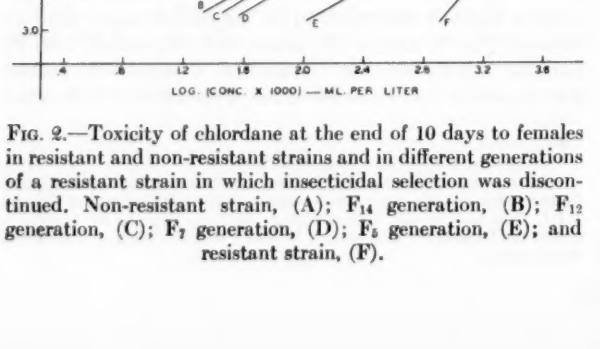

Fig. 2.—Toxicity of chlordane at the end of 10 days to females in resistant and non-resistant strains and in different generations of a resistant strain in which insecticidal selection was discontinued. Non-resistant strain, (A); F_{14} generation, (B); F_{12} generation, (C); F_7 generation, (D); F_5 generation, (E); and resistant strain, (F).

The assumption of homozygosity for resistance or lack of it in the two parental strains was further tested by determining the resistance of progeny from pair matings. The resulting data indicated great variation in resistance among individuals in each strain. These results seem inconsistent with a simple, single-factor hypothesis. Instead, it would be more logical to assume that each strain consists of a multiplicity of phenotypes for resistance, and these probably reflect a multiplicity of genotypes. The fact that there is a slope to the regression lines also suggests varying genotypes in the F_1 individuals for resistance. Barbesgaard & Keiding (1955), working with a strain of house fly resistant to DDT, BHC, and chlordane concluded that several genes were perhaps responsible for the resistance to chlordane.

When the indices of resistance obtained for the three strains included in the pair mating experiment were plotted on graph paper, it was observed that the non-resistant strain had the greatest range of variability and the chlordane-resistant strain the least. The range of the X strain was between those of the two parents, but it was of interest that the X-strain cockroaches were closer to the chlordane-resistant strain than the non-resistant strain. In fact, the X-strain pairs of higher resistance overlapped with the chlordane-resistant pairs of lower resistance, as indicated by the tested resistance of their progeny. Overlapping occurred with both sexes. This suggests the hypothesis that there might be dominance or epitasis involved in some of the resistance factors. A dominant gene for high resistance to DDT has been reported in a strain of the house fly, *Musca domestica*, by Lichtwardt (1956); dominant genes responsible for DDT resistance and nicotine sulfate resistance in *Drosophila virilis* have been reported by Oshima & Hiroyoshi (1956); while Tsukamoto & Hiroyoshi (1956) have presented evidence for incomplete dominancy of resistance to nicotine sulfate in *Drosophila melanogaster*.

Summary.—Further information on the inheritance of resistance to chlordane in the German cockroach, *Blattella germanica* (L.), was obtained by determining the order of resistance of: (a) the F_2 progeny of reciprocal crosses between chlordane-resistant and non-resistant strains, (b) the progeny of pair matings within strains, and (c) cockroaches in successive generations of a chlordane-resistant strain in which selection was discontinued.

The toxicological technique consisted of dipping adults in aqueous suspensions of chlordane at different concentrations and observing mortality up to 10 days. The data obtained are presented in the form of regression lines and as indices of resistance.

The results indicate that many loci are involved in the transmission of resistance to chlordane in the German cockroach. They also substantiate previous indications (Grayson *et al.* 1956) that chlordane resistance is transmitted by the autosomes, with no evidence of maternal

factors or sex-linked inheritance. Some of the evidence suggests that resistance factors exerting some form of dominance might be present.

REFERENCES CITED

Barbesgaard, P., and J. Keiding. 1955. Crossing experiments with insecticide-resistant house flies (*Musca domestica* L.). Vidensk, Meddel. Dansk Naturhist. For. København **117**: 84–116.

Bliss, C. I. 1935. The calculation of the dosage-mortality curve. Ann. Appl. Biol. **22**(1): 134–67.

Grayson, J. M. 1951. Response of the German cockroach to sublethal concentrations of DDT and benezene hexachloride. Jour. Econ. Ent. **44**(3): 315–7.

Grayson, J. M., F. E. Jarvis, Jr., and M. Levitan. 1956. Transmission of resistance to chlordane in the German cockroach. Jour. Econ. Ent. **49**(1): 130–1.

Lichtwardt, E. T. 1956. Genetics of DDT resistance of an inbred line of house fly. Jour. Heredity **47**(1): 11–16.

Oshima, C., and T. Hiroyoshi. 1956. Genetic studies of resistance to DDT and nicotine sulfate in *Drosophila virilis*. Botyu, Kagaku. Bull. Inst. Insect Control **21**: 65–70.

Snedecor, G. W. 1946. Statistical methods. The Iowa State College Press, Ames, pp. 138–41.

Tsukamoto, M., and T. Hiroyoshi. 1956. Further studies on the mode of inheritance of resistance to nicotine sulfate in *Drosophila melanogaster*. Botyu-Kagaku. Bull. Inst. Insect Control **21**: 71–76.

A Method for Rearing the Plum Curculio Under Laboratory Conditions Including Some Biological Observations[1]

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Studies on the biology and control of the plum curculio, *Conotrachelus nenuphar* (Hbst.), have long been hampered by lack of a method for rearing the species under laboratory conditions. An effort to achieve this objective was undertaken by the writer in 1949. The account which follows describes the method developed and reports some pertinent biological observations.

The problem immediately encountered was that the the species locally (Geneva, N.Y.) is single brooded. This characteristic is apparently due to genetic factors, a period of hibernation being required before oviposition occurs. Since the species is multi-brooded in the south a culture was obtained through the courtesy of Dr. C. F. Smith, N. C. State College, Raleigh. The original culture consisting of approximately 25 females was collected from the vicinity of Raleigh, N. C. in August 1949. Two individuals of the original culture produced eggs giving rise to the first laboratory generation. In succeeding generations the proportion of ovipositing females increased until after approximately 10 generations virtually all would produce eggs under standard laboratory conditions. This culture has been maintained continuously to date through approximately 40 generations and the method used has proved practical. To maintain daily emergence of several hundred adults has required approximately an hour per day by one technician.

FOOD.—Green apples approximately 1″ in diameter have been found satisfactory for both adults and developing larvae. One bushel of these may give rise to 10,000 larvae assuming 2000 fruits and 5 larvae per fruit. These fruits are readily available locally when commercial plantings are being thinned, and they can be held satisfactorily in commercial storage. As with mature apples, storage losses are reduced by moving apples into storage promptly after picking. While some losses occur from rot after prolonged storage it has been practical to hold thinnings for a year or until the new crop becomes available. It is likely that storage life of thinning apples could be extended through modified atmospheric storage or the addition of shredded oiled paper as employed in commercial handling of mature apples. Of the major varieties grown locally Wealthy has proved best suited to this use. The shrivelling which occurs when the apples are held at high temperature following storage has no adverse effect on developing larvae, this condition presumably being similar to that of "June drops" in which the species normally develops. Thinning apples have been found to bear heavy insecticide residues even after prolonged storage but these appear to be below the toxic level for this species and removal has not been required. A satisfactory method for removing residues has been described by Allen (1956) who found such a procedure necessary in rearing the oriental fruit moth.

Mature apples are satisfactory as food for adults but when infested by larvae breakdown occurs before completion of larval development.

EXPOSURE OF FRUITS FOR OVIPOSITION.—In order to conveniently expose fruits to females for oviposition, cages were constructed which permitted ready access. These cages, provided with friction type lids, were cylindrical, 6 inches in diameter, 6 inches high and constructed of copper mesh screen. A rack of hardware cloth was fastened 3 inches from the bottom of the cage to support the fruits. In removing fruits the cage is jarred so as to dislodge the beetles causing them to fall to the bottom of the cage. Fruits are exposed in oviposition cages for 24 hours. Approximately 25 apples are added to each cage and sufficient females supplied to insure approximately 5 eggs per apple, a single fruit providing sufficient food for this number of larvae. One female per fruit is the usual ratio. However, the rate of oviposition varies with age as will be discussed later so that a constant ratio of females to fruits cannot be maintained. As the sex ratio is approximately even the females can be selected by chance or in carefully controlled tests by use of the sex characters suggested by Thompson (1932).

LARVAL DEVELOPMENT AND EMERGENCE.—After expo-

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sure in oviposition cages apples are held for the period of incubation and larval development in boxes (1 ft. square and 4 inches deep) provided with hardware cloth bottoms. Upon completion of feeding, larvae desert the fruits and drop directly into soil boxes placed beneath the emergence boxes. Where it is desirable to take records on emergence the larvae are first collected in metal trays and counted before transferring to soil boxes. Where larvae are not permitted to enter the soil directly, precautions must be taken to prevent mortality due to desiccation. Larvae are removed from trays at 12-hour intervals, high humidity maintained and the bottom of the trays covered with paper towels under which the larvae take cover. Larvae hatching in fruits are not cannibalistic even under conditions of limited food supply. Soil boxes are provided with 4 to 5 inches of moist sterilized soil, moisture being maintained by sprinkling with water as needed. Soil high in organic matter is used because of its high moisture holding capacity. The soil boxes, 1 foot square and 6 inches deep, accommodate approximately 1000 larvae each. As a precaution against spread of disease, soil is discarded after using. The incidence of disease under these conditions has been negligible.

The only predator encountered in the course of laboratory rearing was a mite identified by Dr. E. W. Baker, Division of Insect Detection and Identification, U. S. Dept. of Agriculture, as Phytoseiidae, *Garmania bulbicola* (Owdms.). Both adult and immature forms of the predator attacked the larvae. Those severely infested were undersized and sluggish, and too weak to enter the soil. Mortality among those entering the soil was high. The infestation was eliminated by dipping curculio-infested apples or the mature larvae in an aqueous suspension of 50% ovex wettable powder at the rate of 1.2 gms. per quart of water.

EMERGENCE OF ADULTS.—The soil boxes containing mature larvae were provided with screen lids. The adults which later emerged congregated on the screen where they could be collected by hand or with a suction device. In routine rearing emergence of approximately 85% was obtained although higher emergence occured under less crowded conditions and when soil moisture was carefully controlled. Newly emerged adults were placed in screen cages of the type previously described. Several thousand adults were held in a single cage with no apparent ill effect from crowding. The adults readily imbibed water provided in vials supplied with cotton wicks. While the humidity limits tolerated by the adults were not precisely determined, low humidity was found to have an adverse effect particularly at high temperature. Routine laboratory rearing was conducted at relative humidity of 40 to 60% and temperature of 80° F.

DURATION OF LIFE HISTORY STAGES AT 80° F.—In making precise observations on life history stages some modifications in the standard rearing technique were required. Determination of incubation period was based on the observed hatch of eggs deposited over a 12-hour period. For ease of observation eggs were transferred to individual cells in a clear plastic block (Plexiglass). The block was ¼-inch thick having the other dimensions of a standard microscope slide. In each block were drilled 20 cells ¼ inch deep. After transferring eggs to the cells the block was covered with a microscope slide held in place by a rubber band. This technique permitted rapid observation with minimum disturbance. High humidity was maintained by placing the blocks in jars containing moist paper. Eggs were isolated because the newly hatched larvae are cannibalistic when forced to hatch in the absence of food whereas cannibalism does not occur among larvae hatching in fruits.

Table 1.—Duration of life history stages of the plum curculio at 80° F. (±1) under laboratory conditions using green apples as food for larvae.

STAGE	DURATION OF STAGE (DAYS)			NUMBER OBSERVED
	Minimum	Maximum	Mean	
Egg	3⁻	3⁺	3	100
Larval	11	21	13	2448
Pre-pupal	8	16	10	
Pupal	8	14	10	7000
Pre-emerged adult	6	19	8	
Pre-oviposition	7	23	13	1000
Total	43	94	57	

The period of larval development was determined by daily collections of mature larvae as they deserted fruits infested over a 24-hour period, allowance being made for the period of incubation.

In determining developmental stages in the soil it proved impractical to make continuous observations of the same individuals because of the disturbance occasioned by inspection. Instead observations were made on a series of larvae held in 6" flower pots filled with soil. One hundred newly emerged larvae were introduced to each pot and at intervals of one or more days several pots were examined and discarded after recording developmental stages. In this manner progressive development could be projected. Rate of adult emergence was determined from daily collections made from soil boxes to which larvae emerging over a 24 hour period had previously been introduced. Soil moisture was maintained at near optimum to eliminate the retarding influence of deficient soil moisture. The pre-oviposition period was determined by daily inspection of apples exposed to isolated pairs for feeding and oviposition. Results of these studies are summarized in table 1. The incubation period could not be determined with precision using eggs deposited over a 12-hour period. Observations made during the course of laboratory rearing suggest that the duration of stages in the soil are likely influenced by factors other than temperature such as soil type, soil moisture and compactness.

RATES OF FEEDING AND OVIPOSITION.—In considering behavior of the plum curculio two classes of insects are recognized based on oviposition response. These are the northern strain in which oviposition occurs following hibernation and the laboratory strain (progeny of the original southern strain) in which oviposition occurs without regard to hibernation. Comparison was made of the feeding and oviposition rate of these two classes under laboratory conditions. In addition records were taken on females of the hibernated northern strain held in an insectary. The northern strain hibernated females were reared the previous summer from field infested prunes and overwintered in outdoor hibernation cages. Individual insects were confined under inverted jelly glasses sup-

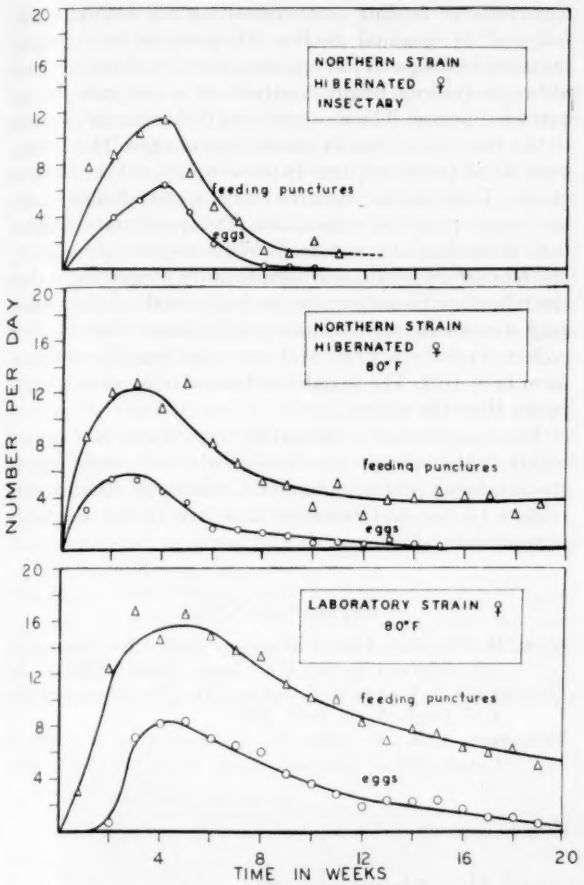

FIG. 1.—The rate of feeding and oviposition for plum curculio of the laboratory and northern strains. Rates are expressed as average daily rate per female per week.

ported on a screen base. The untreated apples provided the insects were removed daily and examined for eggs and feeding punctures with the aid of a binocular microscope. Unlike the species under natural conditions, oviposition punctures did not bear the characteristic crescent-shaped scar and were not readily distinguishable from feeding punctures. All punctures were recorded as feeding punctures assuming that some feeding occurs by the females in excavating the cavity in which the egg is deposited. As a matter of convenience the daily records have been consolidated and are expressed as average daily rate of feeding and oviposition per female per week. These results based on 20 to 35 individuals of each group are shown graphically in figure 1.

Ovipositing females of both strains responded in essentially the same manner maintaining high rates of feeding and oviposition for several weeks followed by gradual decline. A resumption in oviposition did not occur even though individuals continued to feed at a low rate for an extended period. In both groups oviposition was proportionate to feeding.

The oviposition records for the various groups are shown in table 2. There was no significant difference in number of eggs produced by the northern strain under laboratory and insectary conditions. The laboratory strain produced over twice as many eggs as the northern

Table 2.—Record of oviposition by plum curculio of the northern and laboratory strains.

Strain	Eggs per Female			Standard Deviation	Number Observed
	Min.	Max.	Mean		
Northern, 80° F.	57	496	197	102	35
Northern, insectary	12	392	180	108	20
Laboratory, 80° F.	59	1016	489[a]	254	29

[a] Significantly different from other means at 1% level.

strain, this difference being due to higher rate and longer period of oviposition.

Quaintance & Jenne (1912) compiled records of 87 females representing groups from seven widely separated areas and report an average of 145 eggs per female ranging from 1 to 557. The highest production recorded for a single laboratory female was 1016 eggs although complete records are available on relatively few individuals. It seems likely that in the course of laboratory rearing selectivity for most prolific individuals has occurred.

LONGEVITY.—For the purpose of this comparison longevity of the hibernated northern strain was reckoned from time of emergence from hibernation in the spring rather than from emergence as an adult the previous summer. These records are presented in table 3. Females of the laboratory strain were significantly longer lived than hibernated females of the northern strain. Whether this difference is due to the effect of hibernation on the northern strain or to inherent difference in vigor is not apparent. Both classes normally live for a matter of months with little mortality due to unnatural causes. In these tests the insects were supplied water in vials provided with cotton wicks and this may have contributed in part to their vigor with respect to oviposition and longevity.

SIZE.—Comparison was made between weight of the laboratory strain and field collected adults. The field curculios were collected locally from apple and plum trees as the fruits reached sufficient size for oviposition and weighings made immediately. The laboratory curculios were collected at random from a cage containing several thousand adults approximately 3 weeks old. Individuals were confined in a plastic capsule and weights determined using an analytical balance. A summary of these data is given in table 4. The laboratory adults of both sexes were approximately 25% heavier than the field adults. Among both groups the females were approximately 5% heavier than the males. The laboratory adults were more uniform in weight than the field adults. In comparing weights of field-collected and laboratory-reared curculios it is observed that the spread between the lower limits is greater than the difference in maximum weights. This suggests that the adequate food supply under laboratory conditions does not greatly alter the

Table 3.—Longevity of plum curculio females of the northern and laboratory strains at 80° F.

Strain	Days Alive			Standard Deviation	Number Females Observed
	Min.	Max.	Mean[a]		
Northern	47	204	103	49	15
Laboratory	46	290	138	60	29

[a] Means significantly different at 1% level.

Table 4.—Weights of male and female plum curculio of the northern and laboratory strains.

Strain	Sex	Weight in Milligrams			Standard Deviation	Number Observed
		Min.	Max.	Mean[a]		
Northern, field collected	Female	6.9	18.2	12.2	2.21	106
Laboratory	Female	11.0	18.7	15.4	1.80	100
Northern, field collected	Male	5.0	15.1	11.5	1.83	120
Laboratory	Male	10.9	18.6	14.7	1.69	100

[a] All means significantly different at 1% level.

maximum weight of individuals but under field conditions some eggs are deposited in fruits which are too small for full development of the larvae.

SUMMARY.—The plum curculio *Conotrachelus nenuphar* (Hbst.) has been found well adapted to laboratory rearing. A culture obtained from the multi-brooded area in 1949 has been maintained continuously through approximately 40 generations. Green thinning apples are employed as food for both developing larvae and adults. These are readily available locally when commercial plantings are being thinned and a supply can be held in commercial storage for a year or until the new crop becomes available. One bushel of apples may give rise to 10,000 larvae. The life cycle at 80° F. required an average of 57 days.

The feeding and oviposition rate of the laboratory strain was compared with that of the northern strain under laboratory and insectary conditions. The pattern of behavior was essentially the same, both maintaining high rates of feeding and oviposition for several weeks followed by gradual decline. Oviposition was proportional to feeding and having once ceased was not resumed although feeding might continue at a low rate for an extended period. The most striking difference in response of the two strains was in production of eggs. The laboratory strain produced over twice as many as the northern strain. This increase occurred as a result of higher rate and longer period of oviposition. A proportionately higher rate of feeding also occurred. With respect to longevity the laboratory strain was significantly longer lived than the hibernated northern strain. Individuals of the laboratory strain were approximately 25% larger than the field collected northern strain and were significantly more uniform in weight. The females of both strains were slightly larger than the males.

The species under laboratory conditions has proved highly satisfactory for biological and toxicological studies, being prolific, long lived, relatively uniform with respect to size and behavior and having low mortality from unnatural causes.

REFERENCES CITED

Allen, H. W. 1956. Use of immature apples for rearing the oriental fruit moth. U.S. Dept. Agric. ARS-33-24.

Quaintance, A. L., and E. L. Jenne. 1912. The plum curculio. U.S. Dept. Agric. Bull. 103.

Thompson, J. R., Jr. 1932. Sex differentiation of adults of *Conotrachelus nenuphar*. Jour. Econ. Ent. 25: 807-10.

Power Dusting to Control the Sheep Ked[1]

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The sheep ked, *Melophagus ovinus* (L.), the commonest external parasite of sheep in the Rocky Mountain West, presents a difficult problem in control because of the large numbers of infested sheep requiring treatment. In this region woolgrowers graze sheep in bands of 1,000 to 3,000 head, and one outfit may run from 2 to 10 or more bands. Treating several thousand sheep by dipping or spraying, which are the usual methods of controlling the sheep ked, is hazardous, laborious, and costly. The desire of one Wyoming woolgrower to dust his sheep to control the sheep ked led University of Wyoming personnel to assist him in employing the power-dusting method devised by Matthysse (1945) in New York for treating feeder lambs during winter.

Dusting range flocks in Wyoming with 0.5% rotenone, the most efficient dust in Matthysse's tests, gave favorable reductions of parasites compared with reductions on New York feeder lambs similarly treated. But in neither case did the treatments provide eradication. We agreed with Matthysse that the method was not applicable to breeding flocks, where the objective of treatment is eradication and is attainable by dipping. Nevertheless, the advantages of dusting over dipping or spraying range bands of sheep were so worthwhile that tests were initiated to see if an insecticidal dust could be found that would provide eradication.

The tests included nine different dust treatments: 1, chlordane 5%; 2, dieldrin 1%; 3, dieldrin 1.5%; 4, heptachlor 1%; 5, lindane 1%; 6, toxaphene 5%; 7, rotenone 0.5%; 8, a combination of rotenone 0.5% and Lethane 60 10%; and 9, a combination of rotenone 0.5% and BHC, 0.25% gamma isomer. Treatments were usually given after shearing in spring, but some were given in the fall when the sheep bore partly grown fleeces.

METHODS.—Sheep ked-infested farm flocks, averaging around 60 breeding ewes, were used for testing the effectiveness of the insecticidal dusts. All sheep in a flock were dusted with the same insecticide so that comparisons of effectiveness were made among flocks. Some flocks were dusted twice with intervals of 2, 3, or 4 weeks between treatments. The majority of tests were conducted in spring on shorn flocks that had lambed, but several tests were conducted on shorn flocks before lambing.

Results were assessed by comparing the count of keds on 10 sheep, usually 5 lambs and 5 ewes, immediately before treating, with counts of keds on these same sheep, 1, 3, and 6 weeks after treating. In a few tests one or two

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of the selected sheep died, were sold, or could not be found, in which event other sheep of the same flock were substituted for those lost. Some flocks were further examined during either the latter part of October or the first part of November. At this time the lambs and ewes were selected at random for examination.

Toward the end of the testing period, 1951 to 1956, range bands were treated with the most effective dusts and comparative results obtained.

Controls for this study were: 1, the examination of 10 sheep in the manner described above but from untreated flocks; 2, monthly examinations of two farm flocks from 1949 to 1952 to determine seasonal fluctuations in sheep ked populations.

The first procedure furnished control data for the experimental work shown in tables 4 and 5, while the second procedure supplied control data for all other phases of the study. The second procedure demonstrated that sheep ked populations descended during the summer months but that no infestation ever disappeared completely. Populations increased progressively during fall and winter and reached a peak in spring.

A Howry-Berg sheep duster and chute, shown in figure 1, were used to apply the dusts. The machine was equipped with a 7 hp. engine which ran at 3000 revolutions per minute. At this speed the fan ran at 2800 r.p.m. and drove the dust hard against the sheep. The iron dusting chute had eight outlets for the dust. Two were located tandem in the middle of the floor and three in a vertical line, one above the other, on each side.

The way in which sheep were dusted was quite simple. They were gathered and held in a corral, then worked through a squeeze pen, through a panel sheep chute, and finally through the dusting chute. After the sheep were once started, they trailed each other through the curtain of dust at a rate of about 30 per minute or faster.

The chief diluent in all dusts was pyrophyllite (Pyrax ABB), used for the reason that it produced light curtains of dust through which the sheep would trail each other. Adding light motor oil at the rate of 2% to the dusts further reduced fluffiness and helped in encouraging sheep to pass through the dusting chute.

RESULTS.—Of the dusts applied to shorn sheep in spring, 1.5% dieldrin performed best in eradicating infestations of the sheep ked (table 1). This material was applied to nine flocks and in every case the ked was found to have been eliminated 6 weeks after treatment. A subsequent examination of four of these nine flocks in the fall still revealed no infestation. Dieldrin dust at 1% was applied to 6 flocks and the ked was eradicated in 3 of the 6 flocks at the end of 6 weeks.

Of three flocks treated with 5% chlordane, one was free of infestation 6 weeks after treatment, but an examination of this flock in the fall showed the presence of the

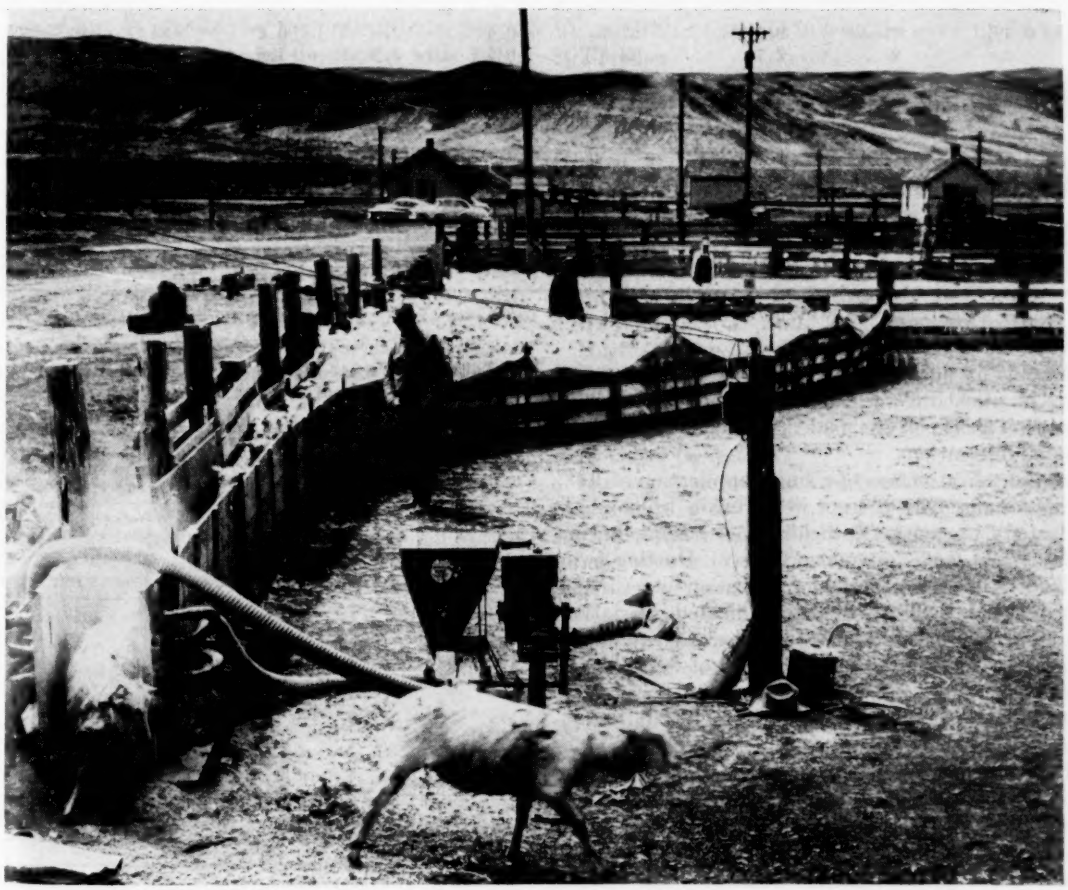

FIG. 1.—Freshly shorn sheep pass through duster for treatment with dieldrin, an effective control for the sheep ked.

Table 1.—Power-dusting sheep after shearing in spring to control sheep keds.

Dust	Test No.	Dusting Rate, Lbs./Min.	Mean Number of Keds per Sheep[a]					Percentage Reduction 6 Weeks After Treating
			Count Before Treating	Count After Treating				
				1 Week	3 Weeks	6 Weeks	Fall	
Dieldrin 1.5%	1	2.4	4.8	0	0	0		100
	2	2.4	66.3	0.8	0.8	0	0	100
	3	2.5	7.1		0.3	0		100
	4	2.5	2.5			0	0	100
	5	2.5	30.5	0.2	0.1	0	0	100
	6	2.6	4.6	0	0	0		100
	7	3.1	21.5	0.3	1.5	0		100
	8	3.1	108.3			0		100
	9	3.3	53.2	0.3	0.5	0	0	100
Dieldrin 1%	1	1.2	203.6	8.9	7.2	7.4		96
	2	1.8	84.0	1.7	1.2	0.4		99.5
	3	2.8	41.6	0.8		0		100
	4	2.8	145.8	2.7	4.1	0.2		99.9
	5	3.0	6.9	0.2	1.2	0		100
	6	3.6	86.6	1.5	0.8	0		100
Chlordane 5%	1	2.3	84.3	3.7	6.8	3.9		95
	2	2.5	11.2	3.5		3.4		70
	3	2.9	18.3	0.5	0.2	0	0.3	100
Toxaphene 5%	1	2.5	18.7	1.7	2.4	2.4		87
	2	2.5	92.9	1.9		4.4		95
	3	3.0	60.1	2.7	9.4	4.5		93
	4	3.2	26.7	3.0	3.8	3.2		88
	5	3.2	61.7	9.7		25.0		59
	6	3.7	67.6	0.1	0.9	0		100
Rotenone 0.5%	1	2.5	37.7	0.4	2.8	0.6		98
	2	2.7	23.5	0.7	5.3	4.8		80
Rotenone 0.5% +Lethane 10%	1	2.5	12.9	2.0	4.0	3.4		74
Rotenone 0.5% +BHC 0.25%	1	2.5	43.3	2.0	11.2	18.1		58
Lindane 1%	1	2.2	57.5	3.4	4.3	4.6		92
Heptachlor 1%	1	2.0	31.0	3.4	5.2	3.6		88

[a] Determined from total counted on 10 sheep.

ked. Among six flocks treated with 5% toxaphene, one was free of the ked 6 weeks after treatment. No fall examination of this flock was made. None of the other dusts eliminated infestations.

Before the effectiveness of a single application of 1.5% dieldrin was discovered, tests were run in which sheep were dusted twice with 1% dieldrin, 5% toxaphene, 5% chlordane, and 0.5% rotenone (table 2). Dusting twice with dieldrin, toxaphene, or chlordane at an interval of 2, 3, or 4 weeks between treatments eradicated infestations with one exception. One out of six flocks treated with toxaphene still was infested at the end of 6 weeks and subsequently in the fall. Dusting of three flocks twice with 0.5% rotenone did not eliminate any infestation.

Tests on power dusting farm and range flocks in the fall of the year with dieldrin, toxaphene, and rotenone-BHC indicated that the ked could not be eradicated with these materials at this season (table 3). But dusting feeder lambs in the fall with either rotenone or dieldrin reduced infestations to very low numbers (table 4).

Table 2.—Power-dusting sheep twice after shearing in spring at intervals of 2, 3, or 4 weeks to control the sheep ked.

Dust	Interval Between Dustings (Weeks)	Mean Dusting Rate Lbs./Min.	Mean Number of Keds per Sheep[a]			Percentage Reduction 6 Weeks After Last Treatment
			Before Treatment	6 Weeks After Last Treatment	Fall	
Dieldrin 1%	2	2.3	68.7	0	0	100
	3	2.3	178.3	0	0	100
	4	2.8	30.8	0	0	100
Toxaphene 5%	2	2.8	2.9	0	0	100
	2	2.9	47.5	0	0	100
	3	2.1	22.0	0.1	2.1	99.5
	3	2.5	49.5	0	0	100
	3		64.0	0[b]		100[b]
	4	2.8	128.9	0	0	100
Chlordane 5%	3	2.8	28.7	0		100
	3	3.7	33.0	0[b]		100[b]
Rotenone 0.5%	2	2.1	46.4	3.2	4.4	93
	3	2.6	78.6	0.1		99.9
	4	2.3	64.8	0.9	1.0	99

[a] Determined from total counted on 10 sheep.
[b] Three weeks after last treatment.

Table 3.—Power dusting sheep in the fall to control the sheep ked.

Dust	Mean Dusting Rate Lbs./Min.	Mean Number of Keds per Sheep[a] Before Treating	3 Weeks After Treating	6 Weeks[b] After Treating	Percentage Reduction at Last Examination
Dieldrin 1.5%	1.8	4.0		0.5	88
	2.3	12.7		8.0	37
	2.4	17.7		16.8	5
	2.4	156.8		52.8	66
Dieldrin 1%	2.3	38.0	5.1	3.7	90
Toxaphene 5%	1.9	27.3	16.1	28.3	None
	2.1	64.2	92.4		None
Toxaphene 5%[c] Rotenone 0.5% +BHC 0.25% g.i.	2.5	40.2	18.0		55
	2.1	11.6		11.1	4

[a] Determined from total counted on 10 sheep.
[b] Approximately 6 weeks.
[c] Applied twice with 3-week interval between treatments.

Results of spring dusting with 1.5% dieldrin were investigated in an unfenced area where untreated sheep gradually infiltrated treated flocks. In the fall of the year, flocks that were treated the previous spring averaged 99% fewer sheep keds than untreated flocks (table 5). The following spring, a year after treatment, ewes averaged 85% and yearlings 72% fewer keds in treated than in untreated flocks. The presence of the ked in increasing numbers on the treated sheep was probably due both to their continued spread from infested sheep and to their ensuing reproduction.

DISCUSSION.—As revealed by the present tests, power dusting of shorn sheep once in the spring with 1.5% dieldrin eliminates infestations of the sheep ked. The method therefore appears suitable for treatment of breeding flocks where the objective is eradication of the parasite. Treated flocks which are not infiltrated by infested sheep remain ked-free not only till fall, as shown in table 1, but all winter. In fact, once sheep are freed of the ked, they do not require retreatment until negligence or some accident allows infested sheep to enter the flock.

The common practice of herding range bands in the West allows infested sheep to gradually infiltrate treated flocks and start new infestations. Hence, to keep populations at low levels, range bands require treatment once each year after shearing in spring.

Range practices leave much room for improvement in the control of sheep ked infestations. If all woolgrowers in a county would treat their sheep, they would reduce the chance of reinfestation. Also, many woolgrowers who do treat, forget to dust newly purchased bucks. New bucks, if parasitized, reinfest the buck herd. Then in December, when the bucks are put in with the ewes, the ked spreads still further.

Table 4.—Power-dusting feeder lambs in the fall to control the sheep ked.

Dust	Mean Number of Keds per Lamb Before Treatment[a]	Three Weeks After Treatment: Mean Number Keds per Lamb[a]	Three Weeks After Treatment: Percentage Reduction
Rotenone 0.5%	74.3	7.3	90
Rotenone 0.5%	146.7	12.9	91
Dieldrin 1.0%	147.4	14.5	90
Untreated	43.5	42.5	2

[a] Determined from total counted on 10 to 15 lambs.

Table 5.—Year-round results of dusting range bands for sheep that were infiltrated gradually by untreated sheep.

Category	Flock Ownership	Mean Number of Keds per Sheep[b] Fall, 1955 Ewes	Fall, 1955 Lambs	Spring, 1956 Ewes	Spring, 1956 Yearlings
Treated	NS	0	0.7	29.5	
	NS	0	0	30.2	
	NS			41.0	74.0
	TS	0.2	0.3	27.9	
	TS			18.7	
	PH			17.9	51.9
	KH	0	0.2		
Untreated	RC	8.6	43.8	181.5	
	EP		27.1		
	EP		25.2		
	SJ			177.7	
	JH			176.2	
	JN				224.5
Mean Treated		0.05	0.3	27.5	63.0
Mean Untreated		8.60	32.0	178.5	224.5
Percentage Reduction		99	99	85	72

[a] Sheep dusted with 1.5% dieldrin in the spring of 1955 after shearing.
[b] Determined from total counted on 10 sheep, except fall count on ewes when only five head were examined for each figure.

The amount of dieldrin dust applied to the sheep by this method is safe for the animals and does not result in the deposition of residues unsafe for consumers of lamb, mutton, and wool (Pfadt & Ryff 1955). Although small amounts of dieldrin are found internally in meat and fat soon after treatment, all traces of it are lost by at least 86 days after treatment. Wool retains dieldrin longer, as small amounts were present for as long as 86 days. However, analyses revealed no traces of the insecticide 191 days after treatment.

Dusting of breeding flocks in the fall was not as successful, at least with the dusts and rates tested. Employed at this time the method never eradicated infestations and reductions ranged from 90% down to no reduction 6 weeks after treatment.

Dusting three pens of feeder lambs in the fall reduced infestations by 90 to 91% 3 weeks after treatment. These results compare favorably with Matthysse's. Two of the groups were dusted with 0.5% rotenone and one group with 1% dieldrin. Although experimental results of Muma *et al.* (1952) and Pfadt *et al.* (1953) showed no benefit from dusting feeder lambs, some stockmen through personal experience believe that when lambs are thin and heavily infested with the sheep ked, dusting is profitable. For those feeders who wish to dust, it appears that Matthysse's recommendation of 0.5% rotenone, pyrophyllite, and 2% light motor oil is best when both effectiveness and absence of toxic residues are taken into account.

There are several advantages in controlling the sheep ked on range bands by power dusting rather than by

spraying or dipping: 1, sheep are not soaked with water. To wet sheep either in spring or fall, which are the practical times for treating, places the animals in a vulnerable condition in the event of a sudden storm. Many sheep in the West have died as a result of being dipped before a cold wave or storm. Even wetting sheep under favorable day-time conditions predisposes them to respiratory ailments because of cold nights; 2, employment of the dusting method usually requires no extra handling of sheep. As sheep leave the shearing pens, they are branded and allowed to accumulate in corrals until all members of a band are sheared. Then they are moved out to the range as a unit. At this point a duster is easily set up and run at the end of the chute through which the sheep pass to the range. Little more time is taken in getting them out of corrals treated than untreated, as sheep go through the dusting chute at a rate of 2000 to 3000 an hour; 3, there is no mishandling or abuse of the sheep, since with the proper squeeze pen and chutes, sheep readily pass through the curtain of dust. No special handling or separation of lambs over several days old needs to be made for the operation. Ewes and lambs pass through the dust almost equally well, though somewhat more slowly than pregnant ewes alone. Newly born lambs must be guided through the dusting chute or be treated by hand; 4, dusting requires fewer personnel than dipping. Three men can easily handle a band of sheep for power dusting. In dipping, additional workers must attend the sheep as they swim through the vat; 5, there is less preparation needed for dusting than for dipping or spraying. There is no need for hauling or accumulating water and for mixing the insecticide with water. From 50 to 100 pounds of 1.5% dieldrin dust treated 1000 head of sheep.

A disadvantage of power dusting is that treatment is limited seasonally to spring after shearing. How soon after shearing the dust must be applied in order to be effective in eradication has not been determined. However, flocks have been dusted as long as 52 and 71 days afterwards with success.

Although dusting of sheep is an easy, simple operation when done properly, it can be laborious and frustrating if done inaptly. Several details should be given attention: 1, use a dust formulated for treating sheep, not crops The right dust will contain pyrophyllite as the main diluent and 2% light motor oil to reduce fluffiness; 2, do not apply excessive rates of dust since this will stop sheep from passing through the chute. Rates of 2.5 to 3 pounds per minute are sufficient; 3, concentrate the sheep in the panel chute, squeeze pen, and adjacent section of the corral before starting the duster engine; 4, do not run sheep against the wind because dense clouds of dust will form in the chute and stop the sheep. Set up chutes in the open, away from buildings so that the wind will blow the excess dust away from operators and sheep coming down the chute. A wind blowing in the direction that the sheep are moving in the chute is most favorable, but a cross wind will do; 5, keep sheep moving in one direction. If sheep turn after passing through the dusting chute and come around to the outside of the corral where the undusted sheep are bunched, the latter will often break away; 6, keep sheep moving without breaks in the line. Sheep pass through the dusting chute readily when trailing one another.

SUMMARY.—Among nine insecticidal treatments applied with a power duster to control the sheep ked, the most effective was 1.5% dieldrin. Treating shorn sheep once in spring with this material eradicated infestations of the parasite. Less effective were dusts of 1% dieldrin, 5% chlordane, 5% toxaphene, 1% heptachlore, 1% lindane, 0.5% rotenone, a combination of 0.5% rotenone and 0.25% gamma isomer of BHC, and a combination of 0.5% rotenone and 10% Lethane 60.

REFERENCES CITED

Matthysse, J. G. 1945. Large scale power dusting of feeder lambs for winter control of the sheep tick. Jour. Econ. Ent. **38**: 285–90.

Muma, M. H., R. E. Hill, E. Hixon, and L. Harris. 1952. Control of sheepticks on feeder lambs. Jour. Econ. Ent. **45**: 833–8.

Pfadt, R. E., L. H. Paules, and G. R. DeFoliart. 1953. Effects of the sheep ked on weight gains of feeder lambs. Jour. Econ. Ent. **46**: 95–99.

Pfadt, R. E., and J. F. Ryff. 1955. Safe use of dieldrin dust for sheep ked control. Jour. Econ. Ent. **48**: 195–8.

The Effect of Erythromycin and Other Antibiotics on the Control of European Foulbrood of Honeybees[1]

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Since the successful use of sulfathiazole to control American foulbrood (Haseman & Childers 1944), many investigators have studied the effects of the newer drugs and antibiotics on bee diseases. Both streptomycin and terramycin have been effective in controlling European foulbrood (Moffett 1954). Pankiw & Jamieson (1956) also report that erythrocmycin was effective in controlling European foulbrood in Canada.

In some parts of the San Luis Valley of Colorado a strain of European foulbrood has failed to respond favorably to treatment with either terramycin or streptomycin. Erythromycin has given encouraging results against this strain in 1954, 1955, and 1956.

METHODS AND MATERIALS.—The colonies tested belonged to commercial beekeepers. The disease had occurred under natural conditions of operation. These experiments were carried out in the apiaries where the disease was found, with a few exceptions in which the colonies were moved to a special apiary.

The colonies were given a disease rating from 0 to 5. The meaning of each rating is given at the top of the next page.

PAID PAPER.
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Rating	*Larvae Affected with European Foulbrood*
0	None
1	Less than 1%
2	Less than 5% and more than rating number 1
3	Less than 10% and more than rating number 2
4	Less than 25% and more than rating number 3
5	25% or more

In 1954 each colony received one-fifth of a gram of cycloserine, hereinafter referred to as MK-65, erythromycin, or tetracycline per treatment. The antibiotic was fed to each colony in a 5-pound honey jar containing sugar sirup. The sirup contained one part sugar and one part water by volume. The other colonies in the 1954 test received equal amounts of plain sugar sirup. Each colony in the aureofac test group was dusted with a mixture containing 7.5 grams of aureofac and 22.5 grams of powdered sugar.

In 1955 one-fifth of a gram of actual erythromycin in the form of erythrocin was dissolved in a pint of water. This solution was sprinkled over the frames of each colony in the erythromycin tests. A streptomycin-penicillin mixture was applied in the same manner. One-sixth of a gram of streptomycin and 50,000 units of penicillin were sprinkled on each colony per treatment. Thirty grams of of penicel was dusted over the frames of each colony receiving this material at each dusting.

In the 1956 tests, gallimycin, which contains 21.1 grams of actual erythromycin per pound, was used as a source of erythromycin. The treatments labeled gallimycin contained the following amounts: #1, 4.5 grams #2, 11.5 grams, and #3 15 grams. Each treatment also had 20 grams of powdered sugar added as a carrier. TM-10 treatment #1 contained 7.5 grams of TM-10 and 22.5 grams of powdered sugar, while treatment #2 consisted of 15 grams of TM-10 and 15 grams of powdered sugar. TM-10 contains 10 grams of terramycin per pound.

Abbott Laboratories supplied the erythromycin, penicel, and gallimycin. Merck & Co. furnished the MK-65. Lederle Laboratories donated the aureofac and tetracycline (achromycin), while Charles Pfizer and Co. provided the TM-10.

Table 1.—The effect of treating colonies diseased with European foulbrood with aureofac, MK-65, tetracycline, erythromycin, and sugar sirup (checks). Martin yard, San Luis Valley, 1954.

Disease Ratings of Colonies Fed:									
Aureofac		MK-65		Tetracycline		Erythromycin		Check	
BT[a]	AT[b]	BT[a]	AT[b]	BT[a]	AT[b]	BT[a]	AT[b]	BT[a]	AT[b]
5	4	5	5	2	0	5	0	2	1
5	2	2	2	5	0	5	5	2	2
4	2	5	5	4	4	2	0	5	5
3	0	5	5	2	0	3	0	3	4
5	2	5	4	5	5	5	0	4	2
1	0	3	3	5	5	2	0	5	5
2	0	2	1	4	0	4	0	1	0
5	5	4	1	1	1	5	2	5	5
		1	5						
30	15	32	31	28	15	31	7	27	24

[a] Before treating.
[b] One week after the feedings were completed.

Table 2.—The effect of erythromycin, a streptomycin-penicillin combination, and penicel, on colonies diseased with European foulbrood. Center, Colorado. 1955.

Disease Rating of Colonies Treated with:							
Erythromycin		Streptomycin-Penicillin		Penicel		Untreated	
Before	After[a]	Before	After[a]	Before	After[a]	Before	After[a]
5	3	5	3	3	5	5	5
5	1	5	5	3	3	5	5
5	2	5	5	5	2	3	5
3	2	5	2	5	5	5	5
5	2	2	0	5	5	3	5
5	1	3	1	5	5	3	2
5	0	5	2	4	5	5	5
3	0	4	0	5	4	4	5
		4	0	2	1	3	2
		4	0	5	5	4	5
36	11	42	18	42	40	40	44

[a] Rating taken 19 days after the colonies were first treated with the antibiotics.

RESULTS.—Forty-one colonies were studied in 1954. These colonies were located in the 60-colony Martin yard, which is 2 miles northeast of Del Norte, Colorado. The colonies were treated three times at weekly intervals, starting on June 16 and ending on June 30.

This apiary had broken down with European foulbrood each summer for several years. Nine colonies were treated with MK-65, while eight colonies were included in the aureofac, tetracycline, erythromycin, and the untreated groups. These colonies were rated for the amount of disease present just before the first treatment, and 1 week after the last treatment (July 7, 1954).

The colonies receiving the erythromycin showed the best recovery from European foulbrood (table 1). Six of the eight colonies entirely eliminated the disease. Four treated with tetracycline, three treated with aureofac, and one of the untreated colonies cleaned out the disease.

In 1955 thirty-eight colonies infected with European foulbrood were borrowed from beekeepers in the San Luis Valley. These colonies were divided into four groups. One group was treated with erythromycin, another with a streptomycin-penicillin combination, the third with penicel, while the fourth was the control group. Penicel was dusted over the frames, but the other materials were dissolved in water and sprinkled over the bees. The colonies were treated three times, starting on June 24 and ending on July 7.

The colonies receiving erythromycin showed the best improvement, as all eight colonies showed some recovery from the disease (table 2). The colonies receiving the streptomycin-penicillin mixture also showed considerable improvement, with the disease rating dropping from 42 to 18. The check colonies showed an increase in disease from 40 to 44. Penicel was ineffective as a control, the disease rating dropping only from 42 to 40. Penicel also was not effective in preventing European foulbrood from developing after inoculating uninfected colonies with diseased brood.

In 1956 one hundred sixty-three colonies which were infected with European foulbrood were tested. The

Table 3.—The results of dusting colonies diseased with European foulbrood with different concentrations of gallimycin and of TM-10 in the San Luis Valley and Fort Collins area of Colorado. 1956.

Treatment	Number of Colonies Tested	Per Cent Reduction of the Disease
Gallimycin No. 1	39	91.5
Gallimycin No. 2	36	84.4
Gallimycin No. 3	34	81.1
TM-10 No. 1	22	77.3
TM-10 No. 2	20	77.7
Control	12	37.5

colonies were located in nine apiaries in the San Luis Valley and in the vicinity of Fort Collins, Colorado.

These colonies were dusted from three to six times, depending on how often the yards could be visited. Most of the colonies treated were from apiaries where the disease had shown some resistance to streptomycin and terramycin in the past. Although previous work has shown that the treatments should be applied weekly for best results, many of the colonies were dusted at 2-week intervals owing to the impossiblity of visiting all the yards within a week.

Three dosages of gallimycin were tested, as well as two dosages of TM-10. Gallimycin contains erythromycin, while TM-10 has about 2% terramycin in its formula. The lowest dosage of gallimycin gave the best control of the disease with a 91.5% reduction occurring (table 3). All three forms of gallimycin-treated colonies had a slightly better recovery rate than the TM-10-dusted colonies. Both the gallimycin- and the TM-10-treated colonies showed much larger percentages of recovery than the untreated colonies.

Gallimycin was less damaging to the weak colonies than TM-10; also there was no noticeable loss of brood in the gallimycin-dusted colonies, while some loss occurred in colonies treated with TM-10.

DISCUSSION.—Seven different antibiotics were tested to determine their effect in helping colonies recover from European foulbrood over a period of 3 years. A total of 242 colonies were included in these tests. Erythromycin (used in the form of gallimycin in 1956) gave the most favorable results among the antibiotics studied.

Previous experience has shown that colonies badly diseased with European foulbrood are so weakened that even if they recover from the disease, they often are too weak to gather surplus honey or live through the winter. The successful control of European foulbrood depends on preventive dustings or feedings in apiaries with a previous history of the disease. Early treatment when the colonies were first breaking down with the disease were more successful than later treatments. However, the badly diseased colonies used in these experiments, furnished valuable information on the relative effectiveness of different antibiotics.

CONCLUSIONS.—Erythromycin was the most effective antibiotic studied in controlling European foulbrood. It gave better results than aurefac, MK-65, tetracycline, penicel, a streptomycin-penicillin combination, or TM-10.

The gallimycin form of erythromycin is an effective form of erythromycin. It was easy to apply, apparently non-toxic to the bees, and was taken readily by them.

The use of gallimycin would be advisable in areas where terramycin and streptomycin have failed to control European foulbrood.

No material gave 100% control of European foulbrood after the colonies became badly infected. Preventive dusting is advisable in areas with a history of European foulbrood. Any apiary showing a few diseased colonies early in the season should be treated preventively.

No applications of dust should be made during the honeyflow in order to avoid any possible chance that an antibiotic might be stored in the honey.

The lowest amount of gallimycin tested (4.5 grams per colony per treatment) gave a slightly higher cleanup than the higher doses (11.5 and 15 grams).

REFERENCES CITED

Haseman, L., and L. F. Childers. 1944. Controlling American foulbrood with sulfa drugs. Missouri Agric. Expt. Sta. Bull. 482.

Moffett, J. O. 1954. The use of antibiotics in controlling European foulbrood of honeybees. Colorado Agric. Expt. Sta. Tech. Bull. 53.

Pankiw, P., and C. A. Jamieson. 1956. Studies on the manifestation, control and prevention of European foulbrood disease in package bee colonies. Abstract from a paper presented at the 10th International Congress of Entomology held in Montreal, Quebec, Canada, August 17–25.

The Attention of All Readers is Called to the Sustaining Associates Listed on Cover iii of this Issue.

Effects of Oil Spray and of Variation in Certain Spray Ingredients on Juice Quality of Citrus Fruits in California Orchards, 1950–1953[1]

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The effect of single applications of oil spray annually, in each of the different months of the year, on juice quality of Valencia oranges in California was reported by Riehl *et al.* (1956). Within the conditions prevailing in the experiment, the results in a single orchard showed a high probability that the reduction in soluble solids of the juice from oil spray could be avoided if applications were made in August and September.

Cressman (1955) stated that no differences were found in two comparisons in which oil spray was applied to Valencia oranges in California in September; whereas lower soluble solids occurred when spray was applied in October or later in the year. Cressman emphasized the fact that the primary objectives of his experiments were not the study of effects on fruit quality, however, and that spray materials, size of plots, and amount of replication varied according to the main purpose of the experiment.

Bartholomew *et al.* (1951) concluded that the juice quality of oranges in California from trees sprayed with parathion is better than that of fruits from oil-sprayed trees; most of the juice tests were with navel oranges, and since the data collected for Valencia oranges showed the same trend the results were reported without designating the respective varieties. (The date of application of the spray may have influenced the observed reductions in soluble solids of the juice by oil spray.)

The effects on citrus of very low concentrations of 2,4-dichlorophenoxyacetic acid (2,4-D) in oil-spray mixture were reported by Stewart & Ebeling (1946) and by Stewart *et al.* (1952). In the latter paper the authors stated that the use of 2,4-D as an amendment for oil spray reduced leaf drop and seemed to influence the percentage of total soluble solids in oranges. Although it was logical to think that leaves held on the tree would contribute to an increase in juice quality, further investigation appeared warranted.

The effects of special narrow-cut petroleum fractions of naphthenic and paraffinic composition on citrus juice quality and leaf drop have been reported by Riehl *et al.* (1954). California spray oils were used as the standard reference treatment, but comparisons with non-oil pesticides or no treatment were not included.

Observations of the effects of several factors of oil spray on the juice quality of citrus fruits in southern and central California during the seasons from 1950 through 1953 are reported in this paper. Determinations of juice quality were given primary consideration in the experimental design. The experiments were planned to evaluate the effects of (1) application in late summer in accord with practices generally recommended for the use of spray oil on citrus trees in southern California, (2) variation in formulation, (3) the addition of 2,4-D to the spray, and (4) the use of three highly paraffinic petroleum oils and an isoparaffinic hydrocarbon oil.

Materials and Methods.—The various oils used and important physical characteristics of each are listed in table 1. Although determinations of the percentage paraffins were not made for paraffinic oils J and K, and the isoparaffinic hydrocarbon oil L, other information indicated relatively high paraffinicity. Oil J was obtained from an East Texas crude and has a viscosity index of 100; oil K was obtained from a mid-continent crude and has a viscosity index of 90. The source of oil I is an eastern crude. The isoparaffinic hydrocarbon oil is a fraction of an oil obtained from the process of catalytic alkylation of low molecular weight olefins; the nature of the production process and the relatively high viscosity for its average molecular weight and fractional distillation range indicate isoparaffinic composition. While the unsulfonated residue (Cox 1943) of the isoparaffinic oil is very low in relation to California citrus spray oils, the neutralization number (mg. KOH/gm. oil) was zero and seemed to be stable for samples that had been stored in cans for intervals as long as a year; in addition, no symptoms of acute injury occurred in preliminary trials with citrus.

Formulations of the oils for use in aqueous spray mixture included proprietary emulsive or flowable emulsion, tank-mix with blood albumin spreader (Smith 1932), and an experimental emulsive formulation with 13 ml. of a mixture of oil-soluble emulsifiers per gallon of oil (the emulsifier mixture consisted of 2 parts of No. 1159 and 1 part of No. A 023 emulsifiers, made by E. F. Houghton and Co., 303 W. Lehigh Ave., Philadelphia 33). The highly paraffinic petroleum oils and the isoparaffinic hydrocarbon oil were supplied in the neat form, but spray mixtures comparable to those for the proprietary spray oils could be obtained by means of the experimental emulsive formulation.

Leaves may remain on a citrus tree for as long as 3 years. When the present experiments were initiated, available evidence indicated that the functions of the leaf might be influenced throughout its life by residual amounts of oil (Rohrbaugh 1934) from a single application. This possibility could be eliminated in part in our experiments because, at the time, there were a few orange orchards which had never been sprayed with oil, and it was possible to obtain one of these in each of the representative climatic districts.

[1] Paper No. 934, University of California Citrus Experiment Station, Riverside. Accepted for publication October 10, 1956.

The pesticide chemicals discussed may not be used unless a tolerance has been established or an exemption from the requirement of a tolerance has been granted for each specific use, irrespective of the information contained in this report.

[2] The writers wish to express their appreciation to the following: to the California Spray Chemical Corporation for determinations of the physical and chemical properties of the California spray oils; to the Shell Oil Company and the Socony-Vacuum Oil Company, Inc., for the highly paraffinic oils and their properties; to the Tide Water Associated Oil Company for the isoparaffinic oil and its properties; to E. F. Houghton and Company for a supply of emulsifiers Nos. 1159 and A 023; to the citrus growers who granted the use of their orchards for experimental plots, to D. L. Lindgren of the University of California Citrus Experiment Station, and to P. D. Gerhardt, formerly of this station, for assistance with the HCN fumigations; and to Mrs. Margaret Larabee Kirby, formerly of the Department of Plant Biochemistry of this station, for valuable assistance in the course of the work.

Table 1.—Important physical characteristics of experimental oils.

Type	Average Molecular Weight	Per Cent Paraffins	Viscosity S.S.U., 100° F.	Temperature, °F., for Distillation: Per Cent 10	50	90	Per Cent Distilled at 636° F.	Per Cent U.R.
California spray oil[a]								
Light-medium grade								
A	284	47.0	67.0	570	623	685	60	92.5
B	263	45.0	72.0	583	622	693	51	93.0
C	273	55.0	67.0	581	626	707	54	94.8
D	260	47.0	72.0	572	617	714	57	92.2
Medium grade								
E	297	47.0	75.7	590	632	687	46	92.5
F	290	45.0	81.3	598	637	702	42	94.0
G	295	55.0	76.5	587	640	726	42	95.6
H	267	46.0	82.3	583	647	737	46	92.0
Highly paraffinic oil								
I	332	77.0	102.5	662	694	Cracked	0	92.0
J	333	—	105.0	608	660	720	30	83.0
K	359	—	115.0	700	Cracked	—	0	88.0
Isoparaffinic oil								
L	258	—	130.0	557	590	651	83	49.2

[a] California Department of Agriculture classification (Erb 1932, Marshall 1932).

Various non-oil pesticides were used in accord with the pest control required in the given orchard. Fumigation with liquid HCN applied under canvas tenting with a vaporizer according to standard commercial procedure in California (Quayle 1941), or a spray of 25% parathion wettable powder, was used for the control of scale insects. The non-oil acaricides used were 50% ovex (Ovotran) in wettable powder and 15% Aramite in wettable powder. In several orange orchards the infestation of pests was such that it was possible to leave plots untreated.

The concentrations of 2,4-dichlorophenoxyacetic acid (2,4-D) and 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) added to oil spray are given in parts per million acid equivalent of the isopropyl esters per total volume of finished spray mixture.

In southern California, experimental blocks were established in Valencia orange orchards near the cities of Riverside and Santa Ana; in navel orange orchards near the cities of Azusa, Redlands, and Riverside; and in lemon orchards near the cities of Riverside, San Fernando, and Ventura. In central California, Valencia and navel orange orchards were selected near Porterville and Visalia. In general, the experimental blocks were rectangular and were several times as long as they were wide, the longer dimension being in the direction of the irrigation. Each plot contained 2 trees, 1 in each of a pair of rows, and treatments were assigned to plots at random in each of 5 replicates.

Orange trees were sprayed once every year, those in central California being treated during the first half of August and those in southern California some time between mid-August and the end of September. Lemon trees were sprayed twice each year, once in the late summer and once in April. Late summer applications to lemon trees in the cooler sections along the coast were made in August but in the interior district applications were made about the end of September in accordance with local practice. In general, moderation of temperatures begins in the interior districts late in September and auspices are favorable for avoiding a "hot spell" during the first few days after treatment.

Sprays were applied manually with conventional orchard spray equipment utilizing a high-pressure reciprocating-type pump operating at 550 pounds pressure and provided with satisfactory agitation in the spray tank (Smith 1932). The proper procedure for mixing in the tank was followed for each of the various spray oil formulations (Ebeling 1950). To insure thorough coverage (Carman 1955) sprays were applied both inside and outside the tree from the ground, and additionally to the outside of the tree from the catwalk of a hydraulic tower. A sufficient volume of material was applied to each tree to wet the foliage to the point of drip. Spraying was done as soon after irrigation as possible (Brock 1929). Applications were not carried out when a period of dry wind from the desert, or when temperatures above the recognized maximum for the district, occurred or were predicted. (The Agricultural Code of California, chapter 1a, section 160.5, authorizes the county agricultural commissioners to set maximum temperatures for oil-spray application in the various localities—usually between 85° and 95° F.)

Tillage, irrigation, fertilization, and other factors of orchard care practiced by the growers were not altered for the experimental blocks. Although these practices varied from one orchard to another, conditions were comparable for the various treatments of each given block.

In addition to the experiments established by the authors, comparisons of oil spray and non-oil pesticides of choice, applied as required, were in progress in three lemon orchards and offered opportunities for juice-

Table 2.—**Valencia orange comparisons in field trials of the effect of oil spray and other treatments, applied for pest control, on the quality of the juice of fruit harvested subsequently.**[a]

Treatment	No. of Comparisons	No. of Differences Between Means Exceeding the L.S.D. Between Means at Odds of 19:1[b]							
		Per Cent Total Soluble Solids		Pounds Soluble Solids/Ton of Fruit		Per Cent Acid		Per Cent Juice	
		+	−	+	−	+	−	+	−
		Southern California							
Non-oil									
Untreated	1	0	1	0	0	0	0	0	0
HCN fumigation	4	0	0	0	0	0	0	0	0
HCN fumigation plus ovex 50% WP at 1 lb./100 gal.	6	1	1	0	0	2	0	0	0
Parathion 25% WP at 2.5 lb./100 gal.	4	1	0	0	0	0	0	0	0
Parathion 25% WP at 2.5 lb./100 gal. (1)[c] and ovex 50% WP at 1 lb./100 gal. (2)	1	1	0	0	0	0	0	0	1
California spray oil									
Spray oil B, C, or D, 1.75%	3	0	0	0	0	0	0	1	0
4 p.p.m. 2,4,5-D in emulsive spray oil A at 1.75%	15	0	1	1	0	0	1	1	0
1.75% Oil A, tank-mix with 4 oz. blood albumin spreader/100 gal.	3	0	0	0	0	0	0	0	0
1.75% Oil A, experimental emulsive	3	0	0	0	0	0	0	0	0
1.75% Emulsive spray oil A (1)[c] and ovex 50% WP at 1 lb./100 gal. (2)	2	0	0	0	0	0	0	0	0
Highly paraffinic oil									
Aver. mol. wt. 332 at 1%, experimental emulsive	1	0	1	0	1	0	1	0	0
Aver. mol. wt. 333 at 1%, experimental emulsive	1	0	0	0	0	0	0	0	0
Isoparaffinic oil									
Aver. mol. wt. 258 at 1%, experimental emulsive	2	0	0	0	0	0	0	0	0
		Central California							
Non-oil									
Untreated	1	0	0	0	0	0	0	0	0
HCN fumigation	1	1	0	1	0	1	0	0	0
California spray oil									
Spray oils B, C, or D 1.75%	3	0	0	0	0	0	0	0	0
2% Flow, emulsion spray oil A	1	0	0	0	0	0	0	0	0
4 p.p.m. 2,4-D in 2% flow. emul. spray oil A	1	0	0	0	0	0	0	0	1
1.75% Oil A, tank-mix with 4 oz. blood albumin spreader/100 gal.	1	0	0	0	0	0	0	0	0
Highly paraffinic oil									
Aver. mol. wt. 332 at 1%, experimental emulsive	1	0	1	0	1	0	0	0	0
Aver. mol. wt. 359 at 0.5% tank-mix with 4 oz. blood albumin spreader/100 gal.	1	0	1	0	0	0	0	0	0
4 p.p.m. 2,4-D, oil aver. mol. wt. 359 at 0.5%, tank-mix with 4 oz. blood albumin spreader/100 gal.	1	0	0	0	1	0	0	0	0
Isoparaffinic oil									
Aver. mol. wt. 258 at 1% experimental emulsive	1	0	0	0	0	0	0	0	0

[a] The standard treatment common to all locations was a proprietary emulsive formulation of light-medium oil "A" at a concentration of 1.75% in aqueous mixture.

[b] Plus (+) or minus (−): higher or lower than the mean of the standard treatment.

[c] Treatments applied as follows: (1) in September; (2) the following March.

Table 3.—Navel orange comparisons in field trials of the effect of oil spray and other treatments applied for pest control on the quality of the juice of fruit harvested subsequently.[a]

Treatment	No. of Comparisons	No. of Differences Between Means Exceeding the L.S.D. Between Means at Odds of 19:1[b]							
		Per Cent							
		Total Soluble Solids		Acid		Ratio Soluble Solids to Acid		Per Cent Juice	
		+	−	+	−	+	−	+	−
Southern California									
Non-oil									
Untreated	1	0	0	1	0	0	0	0	0
HCN fumigation	1	0	0	0	0	0	0	0	0
Parathion 25% WP at 2.5 lb./100 gal.	1	0	0	1	0	0	1	0	0
Parathion 25% WP at 2.5 lb./100 gal., plus Aramite 15% WP at 1 lb./100 gal.	1	0	0	0	0	0	0	0	0
California spray oil									
4 p.p.m. 2,4-D in 1.75% emulsive spray oil A	4	0	1	0	0	0	0	0	0
Flow. emulsion spray oil A at 2%	1	0	0	0	0	0	0	0	0
1.75% Oil A, tank-mix with 4 oz. blood albumin spreader/100 gal.	2	0	0	0	0	0	0	0	0
1.75% Oil A, experimental emulsive	2	0	0	0	0	0	0	0	0
Highly paraffinic oil									
Aver. mol. wt 332 at 0.5%, tank-mix 4 oz. blood albumin spreader/100 gal.	1	0	1	0	0	0	0	0	0
4 p.p.m. 2,4-D, oil aver. mol. wt. 332 at 0.5%, tank-mix with 4 oz. blood albumin spreader/100 gal.	1	0	1	0	0	0	0	0	0
Central California									
Non-oil									
Untreated	1	0	0	0	0	0	0	0	0
California spray oil									
4 p.p.m. 2,4-D in 1.75% emulsive spray oil A	1	0	1	0	0	0	1	0	1
Flow. emulsion spray oil A at 2%	1	0	0	0	0	0	0	0	0
4 p.p.m. 2,4-D in flow. emulsion spray oil A at 2%	1	0	1	0	0	0	0	0	0
1.75% Oil A, tank-mix with 4 oz. blood albumin spreader/100 gal.	1	0	0	0	0	0	0	0	0
1.75% Oil A, experimental emulsive	1	0	0	0	0	0	0	0	0
Highly paraffinic oil									
Aver. mol. wt. 359 at 0.5%, tank-mix, 4 oz. blood albumin spreader/100 gal.	1	0	1	0	0	0	0	0	0
4 p.p.m. 2,4-D plus oil aver. mol. wt. 359 at 0.5%, tank-mix with 4 oz. blood albumin spreader/100 gal.	1	0	1	0	0	0	0	0	0

[a] Same as for table 2.
[b] Plus (+) or minus (−): higher or lower than the mean of the standard treatment.

quality determinations. The plots were relatively large and commercial pest control procedures were used. At one of the locations, there were 6 replicates of the 2 treatments applied alternately to plots consisting of 6 rows of 13 trees each. Each of the other locations consisted of a single replicate of adjacent, comparable plots of 5 acres each.

Sampling for juice-quality determinations followed the procedure given in Riehl *et al.* (1956); results for the various treatments are comparable for each set of samples. Determinations of juice quality were made in accordance with the methods of analysis used by Sinclair *et al.* (1941).

Samples of oranges consisted of mature fruit collected at the time of harvest designated by the packing house according to the usual procedure for the orchard. Lemons, in contrast to oranges, may be picked as often as each month. Samples were taken from the lemon plots in January, April, and September; collection always preceded any spray application. Lemons are segregated into several classes on the basis of rind color (Bartholomew & Sinclair 1951). The "silver" class provides fruit in

a definite stage of maturity which has easily recognized limits. For the present tests, "silver" fruits were defined as those in which both yellow and green were present in the rind in any proportions.

DISCUSSION OF RESULTS.—Analyses of variance were calculated for each juice-quality factor for each set of fruit samples from each experimental block, and, if indicated, the least significant difference between means at the 5% level was derived. Ebeling (1950, p. 210) points out the desirability of the single cultivated block or orchard as the basic experimental unit for plots for comparisons of the effects of pesticide treatments on the physiological function of citrus trees, such as juice quality, size, and yield of fruit. Since the variance may change from one orchard to another, comparisons of differences between treatments occurring in a given orchard should be evaluated in terms of the variance inherent in the particular single cultivated block handled with the given horticultural program.

If differences between treatments were significant at odds of 19:1, each of the means for a given analysis was compared with the mean of the standard treatment (the proprietary emulsive formulation of light-medium grade oil "A" for oranges and of medium oil "E" for lemons, used at a concentration of 1.75% in the finished spray mixture). If the difference was significant, it was tallied and the direction of the change indicated as plus or minus (higher or lower than the mean of the standard treatment). Selected factors of juice quality for each citrus variety are shown in the appropriate table. Results for Valencia oranges are given in table 2, for navel oranges in table 3, and for lemons in table 4.

In general, most of the differences occurred by chance in comparisons of the effect of oil spray vs. non-oil pest control treatments on citrus juice quality at odds of 19:1 It is interesting to consider the magnitude of these differences. Comparisons between treatments were made at the level of the individual experimental block; the effects of non-oil materials were compared with those of the standard oil-spray treatment, the proprietary emulsive formulation of oil "A" for oranges and of oil "E" for lemons used at a concentration of 1.75% in the finished spray mixture. The magnitude of the difference is expressed as a ratio based on oil spray, that is the mean value for the non-oil treatment minus the mean value for the standard oil spray treatment, divided by the mean value for the standard oil spray treatment; the quotient obtained could be expressed as per cent by multiplying it by 100. The direct quotients, or ratios, were arrayed and tallied in class groups of 0.005. These data showed that within the conditions of the experiments about 80% of the differences listed occurred within the range of ratios of minus to plus 0.035, and about 90% within the range of minus to plus 0.05, exclusive of those for the ratio of soluble solids to acid for navel orange; therefore, these respective class groups were used in table 5.

Since most of the differences are not significant it appears that the null hypothesis applies. At odds of 19:1 significant differences in percentage of soluble solids occurred in only 5 out of 16 comparisons for Valencia orange and 0 out of 4 comparisons for navel orange. Differences in percentage acid of lemon juice were significant in 1 out of 15 comparisons, for samples picked in January, in 1 out of 14 for samples picked in April, and in 3 out of 7 for samples picked in September. As shown in table 5, differences for non-oil minus oil spray are both minus and plus; tables 2, 3, and 4 show that this applies also to the significant differences. This situation indicates the need for a more critical evaluation of the effect of oil spray on juice quality and suggests the use of odds of 99:1. At this level, the percentage of soluble solids of Valencia orange juice was significantly higher for non-oil than for oil spray in 1 of 6 comparisons with HCN fumigation and ovex 50% wettable powder at 1 pound per 100 gallons, and in 1 of 4 comparisons with parathion 25% wettable powder at 2.5 pounds per 100 gallons. At odds of 99:1 the percentage of acid of lemon juice was significantly higher for the treatments with various non-oil pesticides, as required and applied under grower-controlled commercial conditions, than for oil spray for 1 of 10 comparisons of samples picked in January and 2 of 5 comparisons of samples picked in September.

Although the number of comparisons is limited, the relative size of the differences and the consistency of the trend indicate mention of the following observation. The maturity ratio of soluble solids to acid of navel orange was consistently lower for fruit from each of the various non-oil treatments applied in August and September than for that sprayed with oil at the same time. The samples of navel oranges for juice-quality determinations were collected when commercial harvest was scheduled for the orchard. In each of the comparisons the maturity ratios of fruit from both the oil-sprayed and the nonoil-treated plots were above the minimum of 8.0 (California Agricultural Code 1953, Division 5, Chapter 2, Section 795.1). The lower values for maturity ratio appear to be associated with higher values for percentage of acid for the non-oil insecticides than for oil-spray. As indicated in table 5, the ratios of the differences between treatments were all larger than −0.05, being −0.136 for untreated, −0.051 for HCN fumigation, −0.059 for parathion, and −0.072 for the combination of parathion and Aramite. Table 3 shows that only one of the differences, that for parathion alone, was significant.

SUMMARY AND CONCLUSIONS.—Observations of the effects of oil spray applied in August and September and of variation of certain ingredients of the mixture and of factors of spraying on the juice quality of citrus fruits in southern and central California orchards during the seasons from 1950 through 1953 are reported.

In general, differences in juice quality between treatments appear either to be attributable to chance or to be associated with the individual orchard for comparisons of oil spray with untreated, HCN fumigation, various sources of California spray oils, various types of spray-oil formulation, and an isoparaffinic oil. Aramite used on lemons only gave similar results.

At odds of 19:1 the percentages of soluble solids of Valencia orange juice were significantly higher for fruit treated with parathion in 2 of 5 comparisons with oil spray; in the remaining 3, values for parathion were higher once and lower twice. For navel orange juice percentages of soluble solids were higher for parathion-treated fruit both times in 2 comparisons with oil spray, but the differences were not significant.

Table 4.—Lemon comparisons in field trials in southern California of the effect of oil spray and other treatments applied for pest control on the quality of the juice of lemons harvested subsequently.[a]

Treatment	No. of Comparisons	No. of Differences Between Means Exceeding the L.S.D. Between Means at Odds of 19:1[b]							
		Per Cent							
		Total Soluble Solids		Acid		Pounds Acid/Ton Fruit		Per Cent Juice	
		+	−	+	−	+	−	+	−
Spray applied in September; fruit samples taken in January									
Non-oil									
HCN fumigation	1	0	0	0	0	0	0	0	0
Aramite 15% WP at 3 lb./100 gal.	1	0	0	0	0	0	0	0	0
Parathion 25% WP at 2.5 lb./100 gal.	2	0	0	0	0	0	0	0	0
Parathion 25% WP at 2.5 lb./100 gal., plus ovex 50% at 1 lb./100 gal.	1	0	0	0	0	0	0	0	0
Various non-oil pesticides as required (grower-controlled test plots)	10	2	0	1	0	0	0	0	0
California spray oil									
8 p.p.m. 2,4-D in 1.75% emulsive spray oil E	2	1	0	0	0	0	0	0	0
4 p.p.m. 2,4,5-T in 1.75% emulsive spray oil E	2	0	0	0	1	0	0	0	0
1.75% oil E, tank-mix with 4 oz. blood albumin spreader/100 gal.	2	0	0	0	0	0	0	0	0
1.75% oil E, experimental emulsive	2	0	0	0	0	0	0	0	0
L.M. emulsive spray oil A, 1.75% parathion 25% WP at 0.5 lb./100 gal.	2	0	0	0	0	0	0	0	0
Highly paraffinic oil									
Aver. mol. wt. 333 at 1%, experimental emulsive	1	0	0	0	0	0	0	0	0
Isoparaffinic oil									
Aver. mol. wt. 258 at 1%, experimental emulsive	4	0	0	1	0	1	0	0	0
Spray applied in September; fruit samples taken in April prior to spring spray application									
Non-oil									
HCN fumigation	1	0	0	0	0	0	0	0	0
Aramite 15% WP at 3 lb./100 gal.	1	0	0	0	0	0	0	0	0
Parathion 25% WP at 2.5 lb./100 gal., plus ovex 50% WP at 1 lb./100 gal.	1	0	0	0	0	0	0	0	0
Various non-oil pesticides as required (grower-controlled test plot)	11	4	1	1	0	0	0	1	0
California spray oil									
8 p.p.m. 2,4-D in 1.75% emulsive spray oil E	2	1	0	0	0	0	0	0	0
4 p.p.m. 2,4,5-T in 1.75% emulsive spray oil E	2	0	0	0	0	0	0	0	0
1.75% oil E, tank-mix with 4 oz. blood albumin spreader/100 gal.	3	0	0	0	0	0	0	0	0
1.75% oil E, experimental emulsive	2	0	0	0	0	0	0	0	0
L.M. emulsive spray oil A 1.75% parathion 25% WP at 0.5 lb./100 gal.	1	0	0	0	0	0	0	0	0
Highly paraffinic oil									
Aver. mol. wt. 333 at 1%, experimental emulsive	1	0	0	0	0	0	0	0	0
Isoparaffinic oil									
Aver. mol. wt. 258 at 1%, experimental emulsive	2	0	0	0	0	0	0	0	0

[a] The standard treatment common to all locations was a proprietary emulsive formulation of medium oil "E" at a concentration of 1.75% in aqueous mixture.
[b] Plus (+) or minus (−): higher or lower than the mean of the standard treatment.

Table 4.—(Continued)

Treatment	No. of Comparisons	No. of Differences Between Means Exceeding the L.S.D. Between Means at Odds of 19:1[b]							
		Per Cent							
		Total Soluble Solids		Acid		Pounds Acid/Ton Fruit		Per Cent Juice	
		+	−	+	−	+	−	+	−
Spray applied in April fruit; samples taken in September									
Non-oil									
Aramite 15% WP at 3 lb./100 gal.	1	0	0	0	1	0	0	0	0
Parathion 25% WP at 2.5 lb./100 gal.	0	0	0	0	0	0	0	0	0
Various non-oil pesticides as required (grower-controlled test plot)	5	1	1	2	0	0	0	0	0
California spray oil									
8 p.p.m. 2,4-D in 1.75% emulsive spray oil E	1	0	0	0	0	0	0	0	0
4 p.p.m. 2,4,5-T in 1.75% emulsive spray oil E	1	0	1	0	1	0	1	0	1
1.75% oil E, tank-mix with 4 oz. blood albumin spreader/100 gal.	1	0	0	0	0	0	0	0	0
Isoparaffinic oil									
Aver. mol. wt. 258 at 1%, experimental emulsive	1	0	0	0	0	0	0	0	0

Table 5.—Observed magnitude of effect of oil spray vs. various non-oil pest control treatments on selected factors of the juice quality of California citrus fruits.

Juice-Quality Factor	No. of Comparisons	Per Cent Comparisons Within Classes of the Ratio of Non-Oil Minus Oil to Oil[a]					
		Below −0.05	−0.05 to −0.035	−0.035 to 0.0	0.0 to +0.035	+0.035 to +0.05	Above +0.05
Valencia Oranges							
Per cent total soluble solids	16	0	6	38	38	6	12
Pounds solids/ton	16	6	6	44	38	6	0
Per cent juice	16	0	6	75	19	0	0
Navel Oranges							
Per cent total soluble solids	4	0	0	25	75	0	0
Ratio soluble solids/acid	4	100	0	0	0	0	0
Per cent juice	4	25	0	25	25	25	0
Lemons							
Per cent acid							
January	15	0	0	60	40	0	0
April	14	0	0	43	43	14	0
September	7	0	0	43	28	14	14
Pounds acid/ton							
January	15	7	20	33	27	13	0
April	14	0	0	43	28	0	28
September	7	0	0	0	57	28	14
Per cent juice							
January	15	13	7	33	47	0	0
April	14	0	0	36	36	0	28
September	7	0	0	43	57	0	0

[a] Mean value for non-oil treatment minus mean value for standard oil treatment divided by mean value for standard oil treatment.

Effects on the maturity ratio of navel orange were not assessed critically; the ratio was lower consistently (apparently because of the higher percentage of acid) for non-oil insecticide treatments on navel orange than for oil-spray treatments. The differences were not significant 3 out of 4 times; the difference was significant for one comparison involving parathion but not for another when Aramite and parathion were used together.

Within the conditions prevailing in citrus orchards in southern California during the experiments, the results obtained show a high probability that reduction in soluble solids of orange juice from oil spray could be avoided if the applications were made in August or September and were in accord with procedures recommended for proper usage.

A similar conclusion is indicated for lemons sprayed twice annually, although the observations suggest that the likelihood of adverse effects of oil spray on lemon juice quality is slightly greater from application in April than in late summer.

The effect of oil spray on citrus juice quality was not influenced by any of four variations of spray oil formulation, but proper procedure for mixing and application are prerequisite.

The addition of 2,4-D to oil-spray mixture as an amendment did not influence juice quality except in the percentage of total soluble solids of navel orange, in which a significant decrease occurred in 3 of 6 comparisons.

Although the highly paraffinic petroleum oils were used at half or less of the concentration of the California spray oil, the percentages of soluble solids were reduced significantly in 3 out of 5 comparisons for Valencia orange and in both of 2 comparisons for navel orange.

Differences in juice quality of citrus fruits were not significant in comparisons of the synthetic isoparaffinic hydrocarbon oil and California spray oil. The contrast in results with the highly paraffinic petroleum oils may be due more to the lower molecular weight of the isoparaffinic oil than to differences in molecular structural configuration of the isoparaffinic oil and highly paraffinic petroleum oils.

REFERENCES CITED

Bartholomew, E. T., and W. B. Sinclair. 1951. The lemon fruit, its composition, physiology, and products. Univ. California Press, Berkeley and Los Angeles.

Bartholomew, E. T., W. S. Stewart, and G. E. Carman. 1951. Some physiological effects of insecticides on citrus fruits and leaves. Bot. Gaz. 112(4): 501–10.

Brock, A. A. 1929. Oil spray damage to citrus. California Dept. Agric., Monthly Bull. 18(10): 572–3.

Carman, G. E. 1955. Spray coverage. Calif. Citrog. 40(12): 440, 462–7.

Cox, A. J. 1943. Terminology of insecticides, fungicides and other economic poisons. Jour. Econ. Ent. 36(6): 813–21.

Cressman, A. W. 1955. Effect of oil and parathion sprays on soluble solids of oranges. Jour. Econ. Ent. 48(2): 216–7.

Ebeling, Walter. 1950. Subtropical entomology. Lithotype Process Company, San Francisco.

Erb, L. W. 1932. California Dept. Agric., Div. Chem. Spec. Publ. Chart. No. 4, California State Printing Office, Sacramento.

Marshall, W. G. 1932. Economic poison decisions. *In* Economic Poisons, 1931–32. California Dept. Agric. Spec. Publ. 116: 7–8.

Quayle, H. J. 1941. Insects of citrus and other subtropical fruits. Comstock Publishing Company, Inc., Ithaca, New York.

Riehl, L. A., E. T. Bartholomew, and J. P. LaDue. 1954. Effects of narrow-cut petroleum fractions of naphthenic and paraffinic composition on leaf drop and fruit juice quality of citrus. Jour. Econ. Ent. 47(1): 107–13.

Riehl, L. A., R. T. Wedding, and J. L. Rodriguez. 1956. Effect of oil spray application timing on juice quality, yield, and size of Valencia oranges in a southern California orchard. Jour. Econ. Ent. 49(3): 376–82.

Rohrbaugh, P. W. 1934. Penetration and accumulation of petroleum spray oils in the leaves, twigs, and fruit of citrus trees. Plant Physiol. 9(4): 699–730.

Sinclair, W. B., E. T. Bartholomew, and Walter Ebeling. 1941. Comparative effects of oil spray and hydrocyanic acid fumigation on the composition of orange fruits. Jour. Econ. Ent. 34(6): 821–9.

Smith, R. H. 1932. The tank-mixture method of using oil spray. California Agric. Expt. Sta. Bull. 527.

Stewart, W. S., and Walter Ebeling. 1946. Preliminary results with the use of 2,4-dichlorophenoxyacetic acid as a spray-oil amendment. Bot. Gaz. 108(2): 286–94.

Stewart, W. S., L. A. Riehl, and L. C. Erickson. 1952. Effects on citrus of 2,4-D used as an amendment to oil sprays. Jour Econ. Ent. 45(4): 658–68.

OBITUARY

Lloyd W. Ford
1919-1957

The many friends and associates of Lloyd Ford were shocked and saddened to learn of his untimely death on February 18, 1957, in an Oklahoma airplane accident. Lloyd was flying a new plane from the factory to his home in El Centro, California. Circumstances not yet fully explained caused the fatal crash.

A native of Colorado, he received his B.S. in entomology at Colorado A & M. He served in the 168th Field Artillery from 1941 to 1945. His tour of duty included active participation in the Philippine theatre. He was honorably discharged as a major.

In 1947 Lloyd came to the Imperial Valley of California, and in partnership with James Vedder, founded the Visco Company, which engaged in airplane dusting and spraying operations. His outstanding ability to inspire confidence and trust rapidly brought his organization to the point where four Colorado A & M entomologists were associated with him in providing scientific counsel to the growers in the area.

Surviving are his wife Margaret—daughters Beverly 12, Debora 5 and son Donald 8. Lloyd was a member of the Entomological Society of America, Alpha Zeta Honorary, B.P.O.E. and President of Oasis Chemical Company.

Here was a man his friends were proud to know.

ED H. LITTOOY

The Systemic Behavior of *O,O*-Diethyl *S*-2-(Diethylamino)ethyl Phosphorothiolate and Its Salts[1]

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The compound *O,O*-diethyl *S*-2(diethylamino)ethyl phosphorothiolate has recently been described by Ghosh & Newman (1955) as an efficient contact and systemic insecticide. This compound,

$$(C_2H_5O)_2\overset{\overset{O}{\|}}{P}SCH_2CH_2N_2(C_2H_5)_2,$$

has an obvious structural relationship to the thiol-isomer of demeton,

$$(C_2H_5O)_2\overset{\overset{O}{\|}}{P}SCH_2CH_2SC_2H_5,$$

but has the important difference that because of the tertiary amine structure it is moderately basic ($K_b \cong 6\times10^{-7}$ at 25° C.) and thus readily forms salts with a variety of strong acids. The monohydrogen oxalate salt of this compound is currently undergoing extensive evaluation as an insecticide and acaricide under the code designation R-6199.

It is the purpose of this paper to evaluate the systemic behavior of *O,O*-diethyl *S*-2(diethylamino)ethyl phosphorothiolate, hereafter designated as thiol-isomer base; of *O,O*-diethyl *O*-2(diethylamino)ethyl phosphorothionate, hereafter designated as thiono-isomer base; and of the thiol-isomer salts, especially the oxalate, hereafter described as thiol-isomer oxalate. The studies reported were largely carried out using P^{32}-labeled compounds, and in some instances comparisons were made with P^{32}-labeled demeton thiono- and thiol-isomers.

Materials and Methods.—The preparation of radioactive P^{32}-labeled *O,O*-diethyl *O*-2(diethylamino)ethyl phosphorothionate (thiono-isomer base) from neutron-irradiated red phosphorus is described elsewhere (Fukuto & Stafford 1956). The product is quantitatively isomerized by heating to 120–180° C. for 2.5 hours to give *O,O*-diethyl *S*-2(diethylamino)ethyl phosphorothiolate (thiol-isomer base). Both isomers were distilled in the falling-film still under high vacuum to give the pure compounds with a relative activity of 8.09 c.p.s. per microgram by end-window Geiger tube, and 12.31 c.p.s. per microgram by windowless flow counter.

The thiol-isomer base readily formed tertiary ammonium salts upon admixture in dry ether with equimolar quantities of strong organic acids such as oxalic, picric, *p*-toluenesulfonic, and phthalic, and a quaternary ammonium salt with dimethyl sulfate. The thiol-isomer oxalate, which was the principal salt used in these studies, was a white crystalline compound m.p. 93–5° C., with a relative activity of 6.06 c.p.s. per microgram by end-window Geiger tube and 9.49 c.p.s. per microgram by flow counter. The preparation of the P^{32}-labeled demeton isomers has been described previously (Fukuto & Metcalf 1954). The methods employed for the investigation of radiotracer preparations of systemic insecticides have been described in detail (Metcalf & March 1952, Metcalf *et al.* 1954).

Discussion of Results.—*Penetration and Translocation from Applications to Roots of Lemon Seedlings in Water Cultures.*—One lemon seedling about 40 cm. high was grown in each of two water-culture solutions containing P^{32}-labeled thiol-isomer base or thiol-isomer oxalate as indicated in table 1. At the intervals given, one young terminal leaf was removed from each plant and 0.3-gm. portions were cut from the tips, homogenized, and assayed for radioactivity. The remainders of the leaves were placed in Munger cells and each cell was infested with 20 adult citrus red mites, *Metatetranychus citri* (McG.).

The data in table 1 indicate a progressive increase in amount of radioactivity present in the leaves with time

Table 1.—Accumulation of radioactive compounds in leaves of lemon seedlings growing in water cultures containing 2.2×10^{-4} M (0.0059%) thiol-isomer base or thiol-isomer oxalate.

Period Preceding Assay	Micrograms of Free Base per Gram of Leaf		48-Hour Per Cent Mortality of Citrus Red Mites	
	Thiol-isomer Oxalate	Thiol-isomer Base	Thiol-isomer Oxalate	Thiol-isomer Base
24 hours	0.8	8.2	50	65
3 days	0.8	12.4	85	100
6 days	20.0	24.0	—	—
16 days	202.0	248.0	—	—

and show the accumulation of the thiol-isomer base and oxalate in amounts which were lethal to the test animal within 48 hours. The rate of uptake from solution was somewhat greater for the free base, especially for the first 3 days. For the first 6 days the rates for both compounds were lower than those previously obtained with schradan and the thiono isomer of demeton from solutions containing equivalent quantities of tagged material. On the sixth day the inhibition of fly-brain cholinesterase was 100 per cent from 10 mg. of leaf tissue and 89.5 per cent from 1 mg. of leaf tissue from the plant treated with thiol-isomer base. The cholinesterase inhibition was 92.6 per cent from 10 mg. of leaf tissue from the plant treated with thiol-isomer oxalate.

Penetration and Translocation from Applications to Stems of Cotton and Lemon Seedlings.—The thiol-isomer base and its oxalate were topically applied to the basal stems of cotton plants at 10 mg. per plant and similarly to lemon seedlings at 20 mg. per plant, with three replica-

[1] Paper No. 935, University of California Citrus Experiment Station, Riverside, California. Accepted for publication October 17, 1956.

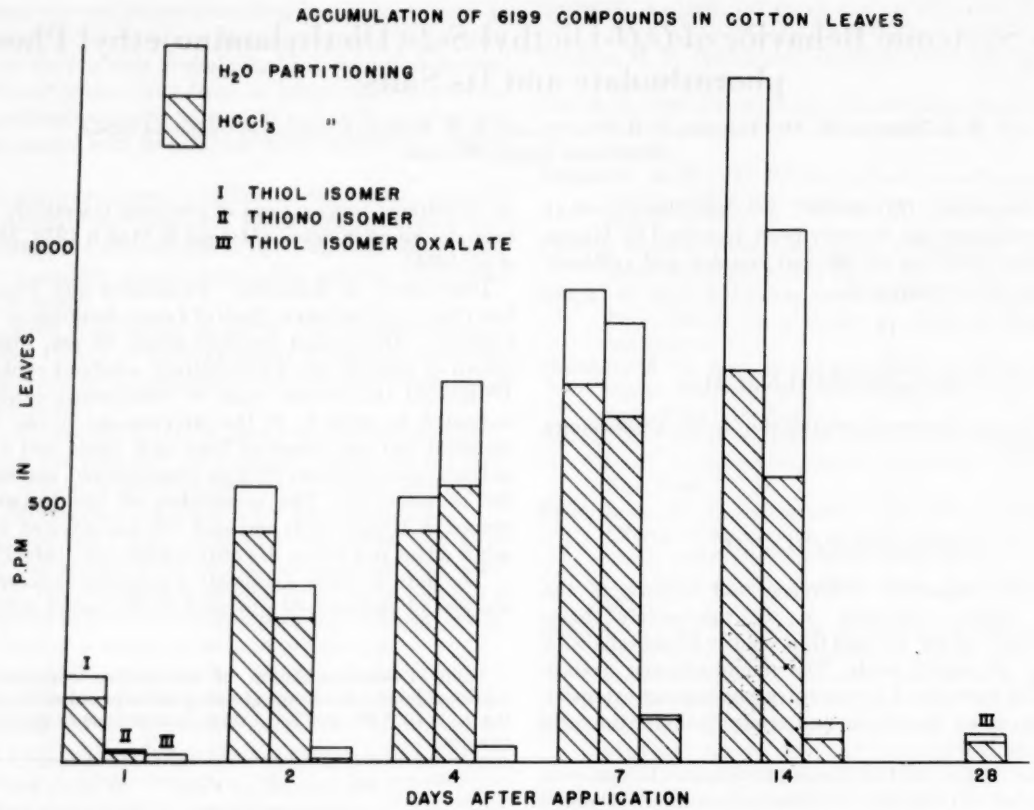

FIG. 1.—Comparative rates of accumulation and decomposition of thiol-isomer base, thiono-isomer base, and thiol-isomer oxalate in cotton leaves after topical application of 10 mg. P^{32}-labeled compounds to basal stem.

tions per treatment. A single lemon plant was treated with the thiono-isomer base, which was available in limited amount. The thiol- and thiono-isomer bases were applied as the pure liquids, and the oxalate was applied as a water solution containing 10 mg. of free base equivalent in 20 microliters. At the intervals indicated in figures 1 and 2, the average radioactivity present in mature leaves was measured by assaying homogenates of equal-sized pieces from each of the replicates, and measuring the corresponding $HCCl_3/H_2O$ partition ratios.

The data in figures 1 and 2 show a progressive increase in the amount of radioactivity in both cotton and lemon leaves. The thiono- and thiol-isomer bases accumulated at about the same rate, but the thiol-isomer oxalate was taken up at an average rate of about 0.1 that of the free base. This is shown graphically in the radioautographs of figure 3. In both cotton and lemon leaves there was a progressive degradation of the insecticides as indicated by the increasing proportion of water-soluble materials (upper clear portion of histograms). However, over the 30-day period of the experiment more than half of the material accumulating in the leaves was still intact ester.

The translocated material from the topical applications to the stems of cotton plants at 10 mg. per plant was rapidly lethal to the two-spotted spider mite, *Tetranychus telarius* (L.), and the greenhouse thrips, *Heliothrips haemorrhoidalis* (Bouché), as shown in table 2. The thiol-isomer base, as would be expected from its much greater rate of translocation, was more rapidly effective than the thiol-isomer oxalate when the two were applied at equal rates.

Both the thiol-isomer base and the thiol-isomer oxalate are extremely effective inhibitors of the cholinesterase enzymes of nerve and muscle. The thiol-isomer oxalate in *in vitro* experiments had an I_{50} value for fly-head cholinesterase of 5×10^{-9} M, compared to 3×10^{-8} M for the thiol-isomer base and 3×10^{-6} M for the thiono-isomer base. This latter degree of inhibition probably results from traces of thiol-isomer base which are readily formed

Table 2.—Toxicity of translocated systemics to the two-spotted spider mite *Tetranychus telarius* (L.) and the greenhouse thrips *Heliothrips haemorrhoidalis* (Bouché) feeding on leaves of cotton plants topically treated on stems with 10 mg. of compound.

Period Preceding Assay	24-Hour Per Cent Mortality	
	Mites	Thrips
24 Hours		
Thiol-isomer base	90	0
Thiol-isomer oxalate	70	30
Demeton thiol isomer	100	60
48 Hours		
Thiol-isomer base	95	80
Thiol-isomer oxalate	100	100
Demeton thiol isomer	100	100

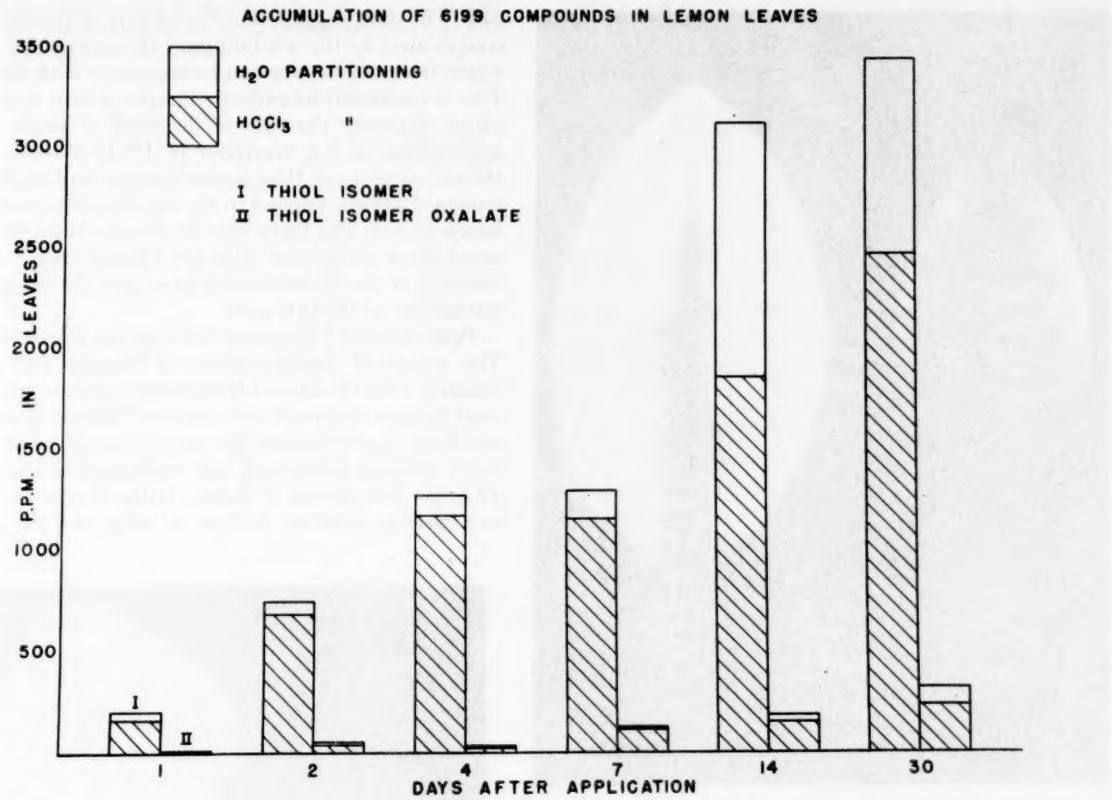

FIG. 2.—Comparative rates of accumulation and decomposition of thiol-isomer base and thiol-isomer oxalate in lemon leaves after topical application of 20 mg. to basal stem.

by isomerization (Fukuto & Stafford 1956). Thus the cholinesterase inhibition serves to detect amounts of the thiol-isomer base as low as 0.001 p.p.m. This method was utilized to follow the translocation of these compounds in cotton leaves by adding known amounts of leaf homogenates to Warburg vessels containing fly cholinesterase and acetyl choline, as shown in table 3. The data show the increase in toxicant with time and the preferential translocation to new leaves.

It was of interest to compare the rate of uptake of the thiol-isomer base into cotton leaves with that of demeton thiol isomer from applications made at the same dosage and carried out at the same time under equivalent conditions. These are reported in detail elsewhere (Metcalf *et al.* 1956). Plots of the rate of increase of the toxicants in the leaves over the initial 7-day period yield fairly good straight lines and show that the demeton thiol-isomer accumulated in the leaves about 3.2 times as rapidly as the thiol-isomer base.

Cotton stems, especially of young plants, were severely injured by the application of the thiol-isomer base, and upon recovery produced gall-like growths at the point of injury. Treated lemon seedlings, however, showed no injury.

A series of salts of the radioactive thiol-isomer base were prepared and applied to the stems of cotton plants as outlined above. After intervals of 8 and 16 days a mature leaf was removed from each plant, homogenized, and assayed for total radioactivity, as shown in table 4. It appears that the degree of injury produced by the treatment is correlated with the speed of penetration.

Penetration into Lemon Leaves.—The top leaves of young lemon seedlings were dipped into water solutions of the P^{32} thiol-isomer base, several of its salts, and demeton thiol isomer at 3.7×10^{-3} M, with 0.01 per cent Triton X-100 wetting agent. The rates of penetration were measured at intervals by stripping the surface of 2 to 6 leaves in water and measuring the radioactivity in an aliquot, then comparing this with the radioactivity in an aliquot of the total leaf homogenate after stripping. The results are given in table 5 and indicate that the thiol-

Table 3.—Inhibition of fly-head cholinesterase by cotton leaf tissues after topical application of 10 mg. thiono- or thiol-isomer base to stem.

TREATMENT	PER CENT INHIBITION AFTER TREATMENT			
	2 Days		5 Days	
	10 mg. tissue	*1 mg. tissue*	*10 mg. tissue*	*1 mg. tissue*
Thiono-isomer base				
New leaf	4	0	70	0
Mature leaf	13	0	—	—
Thiol-isomer base				
New leaf	93	90	—	96
Mature leaf	51	4	92	84

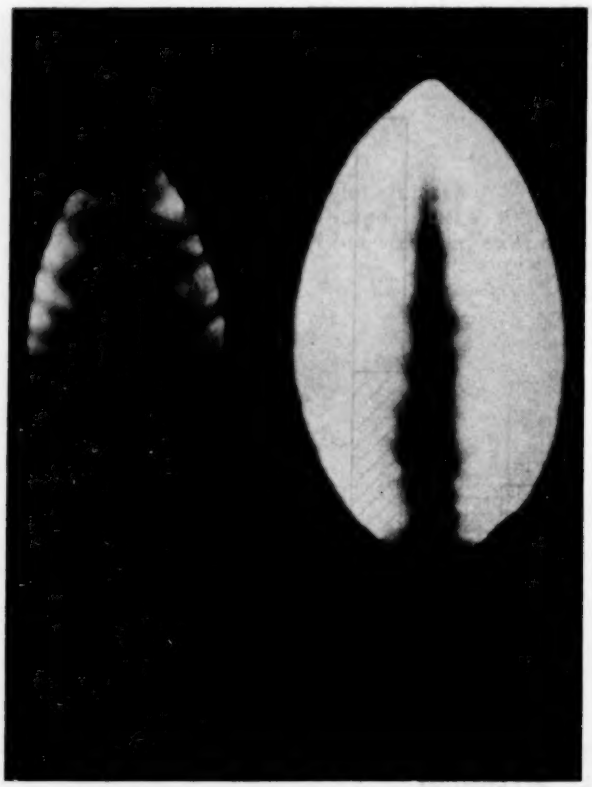

FIG. 3.—Radioautographs showing relative rates of accumulation of P^{32} thiol-isomer base (at right) and P^{32} thiol-isomer oxalate (at left) in lemon leaves 14 days after topical applications of 20 mg. to base of lemon stem.

isomer base penetrates rapidly and is largely retained after washing, within 6 hours. The thiol-isomer oxalate penetrates much more slowly and from other evidence may only become fixed in the superficial layers of the cuticle from which it cannot be removed by rinsing.

The various thiol-isomer base salts showed rather variable penetration and at 144 hours the following values for percentage penetration were obtained: oxalate—34.2, citrate—43.3, *p*-toluene sulfonate—33.5; hydrochloride—34.6. The data suggest that the picture of penetration is complicated by the solubilities of the compounds in leaf waxes from which they can be separated with difficulty. This is confirmed by radioautographs shown in figure 4, which illustrate the rate of dispersal of single topical applications of 0.1 microliter of P^{32} thiol-isomer base, thiono-isomer base, thiol-isomer oxalate, and thiol-isomer dimethyl sulfate, applied to the upper central surfaces of lemon leaves. The figure clearly demonstrates the ready penetration and spread of the thiol-isomer base, the lesser mobility of the thiono-isomer base, and the comparative immobility of the two salts.

Table 4.—Accumulation of radioactive compounds in leaves of cotton plants treated topically at base of stems with 10 mg. thiol-isomer salts.[a]

Thiol-Isomer Salt	Radioactive Compounds γ/g. of Leaf After 8 Days	After 16 Days	Degree of Injury
Trichloroacetate	68	652	Dead tissue wherever salt was applied
Picrate	65	378	About ⅓ as much dead tissue as by trichloroacetate above
Oxalate	132	242	Stem lesions (20)
Dimethylsulfate	29	209	Stem lesions (11)
Phthalate	73	54	Stem lesions (11)
Citrate	3	20	None
p-Toluene sulfonate	7	5	None

[a] Solutions contained 10 mg. of salt per 20 μl.

Penetration of Thiol-isomer Oxalate into Citrus Fruits.—The extent of contamination of oranges and lemons sprayed with P^{32}-labeled thiol-isomer oxalate was measured by spraying fruit on a mature Valencia orange tree and on a Eureka lemon tree to the run-off point with a water solution containing the compound at the rate of 40 g. per 100 gallons of water. Triton X-100 was added as a wetting agent at the rate of 15 g. per 100 gallons.

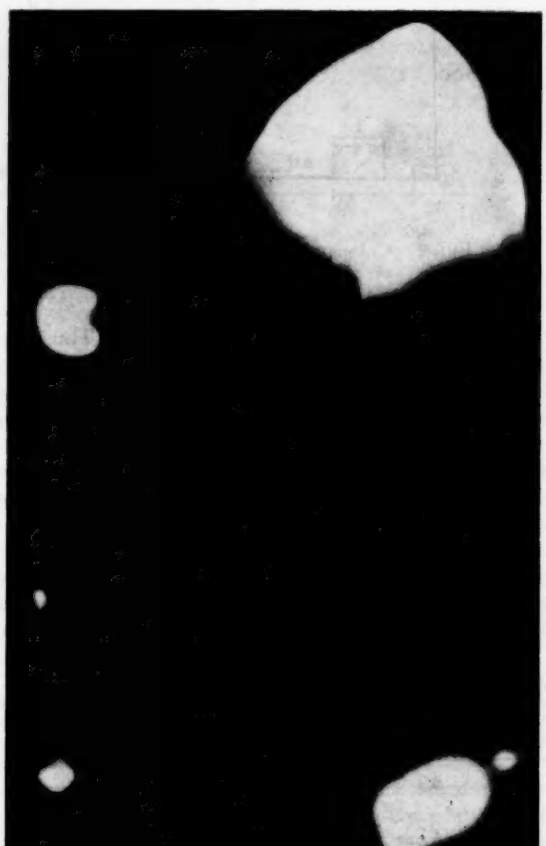

FIG. 4.—Radioautographs showing dispersal of P^{32}-labeled compounds 14 days after topical application of 0.1 microliter to centers of lemon leaves. Upper left: thiol-isomer oxalate; upper right: thiol-isomer base; lower left: thiol-isomer methosulfate; lower right: thiono-isomer base.

Table 5.—Penetration of thiol-isomer base and its oxalate into lemon leaves dipped into 3.7×10^{-3} M solution containing 0.01 per cent wetting agent.

Compound	Hours After Treatment	γ per Gram of Leaf		Per Cent Penetration
		On Surface	In Homogenate	
Thiol-isomer base	1	99	48	32.6
	6	33	104	76.7
	24	27	50	65.0
	48	20	145	87.9
	120	27	119	81.6
	142	21	124	85.6
	264	23	94	80.3
Thiol-isomer oxalate	1	100	26	20.6
	6	136	50	26.9
	24	113	84	42.6
	48	83	100	54.7
	120	79	125	61.2
	192	65	175	76.1
	264	75	135	64.2
Demeton thiol isomer	1	77	25	23.5
	6	23	13	36.8
	48	19	17	48.2
	96	26	35	57.3
	144	20	36	64.0

The dosage used was that considered as suitable for commercial practice.

Replicates of 10 fruits were picked from each tree at the intervals indicated in table 5. The surface contamination was determined by stripping the fruit with water containing 0.1 per cent Triton X-100 and assaying aliquots of the combined strippings. The washed fruits were carefully peeled to avoid any possibility of contaminating the pulp. The peel and pulp were minced separately and radioassays were carried out on weighed samples either evaporated in planchets (3 and 7 days) or extracted by the method of Diggle and Gage (1955) (14 and 28 days). This involved the extraction of the fruit samples with 67 per cent acetone, removal of the acetone, and extraction of the residue at pH 11 in benzene. The benzene solution was concentrated in the Kuderna-Danish evaporative concentrator for radioassay or was acidified and extracted with water for comparative enzymatic assay with human red-cell cholinesterase by the method of Diggle & Gage (1955).

The values obtained in this study are presented in table 6 and show that the residues in the pulp of both fruits were below the limits of detection of either the radioassay or the cholinesterase assay methods. The green lemon fruits, which were considerably smaller, contained consistently higher peel residues than the ripe Valencia oranges. Remarkable agreement was found between the radioassay and enzyme assay.

Cotton-Seed Treatment.—The P^{32}-labeled thiol-isomer base, thiol-isomer oxalate, and demeton thiol isomer were topically applied to acid-delinted cotton seeds (average weight 100 mg.) at a dosage of 2 mg. per seed. The thiol-isomer oxalate was used in saturated water solution. After standing overnight the seeds were planted in 6-inch pots. At the intervals indicated in table 7, portions of the young plants were homogenized and the total radioactivity and $HCCl_3/H_2O$ partition coefficients were determined. The data show that the demeton thiol isomer accumulated in the cotyledons much faster than the thiol-isomer base or the thiol-isomer oxalate and that in general this relationship held for the accumulation of the three materials in the first and second true leaves. The rate of accumulation is influenced by the water solubility of the compounds and suggests that the thiol-isomer oxalate and thiol-isomer base may be leached away from the seed by irrigation of the pots. The partition coefficients at 39 days indicate a considerable breakdown of the compounds into inactive water-partitioning compounds.

Table 6.—Residues of thiol-isomer oxalate in orange and lemon fruits after spraying with 40 g. per 100 gallons.

Time After Spraying	Residue, P.P.M.					
	Orange			Lemon		
	Surface	Peel	Pulp	Surface	Peel	Pulp
1 Hour	0.28			0.225		
3 Days	0.15	0.063	0.00	0.217	0.24	0.00
7 Days	0.317	—	0.00	0.38	0.21	0.00
14 Days	0.15	0.078 (0.06)[a]	0.00	0.21	0.32 (0.34)[a]	0.00
28 Days	0.033	0.062 (0.05)[a]	0.00	0.035	0.24 (0.30)[a]	0.00

[a] Values in parentheses obtained by enzymatic assay by Dr. J. C. Gage.

The demeton thiol-isomer caused a marked injury to the distal margins of the cotyledons, and the growth of these plants was at first somewhat slower than that of plants from seeds treated with the thiol-isomer base or thiol-isomer oxalate. The true leaves showed no injury from any treatment. The newly emerged cotton plants were grown in a greenhouse room where they were exposed to the attacks of the flower thrips, *Frankliniella tritici* (Fitch). Check plants were nearly defoliated, but the plants from the treated seeds remained free of injury for from 2 to 5 weeks, with the order of protection being: thiol-isomer oxalate < thiol-isomer base < demeton thiol isomer.

Paper Chromatography.—The two systems of reverse-phase paper chromatography used by Metcalf *et al.* (1954) for demeton isomers and plant metabolites were tested with P^{32}-labeled thiol-isomer base and its oxalate. The thiol-isomer base streaked badly on propylene-glycol-treated paper, while the oxalate was moved very little by

Table 7.—Translocation of thiol-isomer base, thiol-isomer oxalate, and demeton thiol isomer into cotton plants after seed treatment.

Compound	Radioactivity in Leaves in γ per Gram[a]					$HCCl_3/H_2O$ Partition 39 Days
	Cotyledons	1st True Leaves		2nd True Leaves		
	18 Days	19 Days	25 Days	33 Days	39 Days	
Thiol-isomer base	221	457	43	25	20	0.7
Thiol-isomer oxalate	27	5	8	7	4	1.0
Demeton thiol isomer	13,810	91	149	53	107	2.9

[a] Calculated as material applied.

the *n*-hexane and toluene solvent. Streaking also occurred on paper treated with silicone 550,[2] when the upper phase of a mixture, by volume, of 10 parts chloroform, 10 parts absolute ethanol, and 6 parts of water was used as a solvent. Less streaking occurred when veronal buffer pH 9.4 was used to prepare the solvent.

Several systems were tested, but the sharpest spots for the thiol-isomer base and its oxalate were obtained by using untreated Whatman No. 1 paper and a solvent mixture of *n*-hexane 14 parts, chloroform 6.5 parts, methanol 8 parts, and 6 N HCl, 0.4 parts. With this system, chloroform extracts of cotton leaves from plants treated with the radioactive systemics showed only single spots with R_F values identical with those of the pure compounds. Similar results were obtained with paper electrophoresis of the chloroform extracts of treated cotton leaves, using a buffer of 0.1 N HCl with a potential of 400 volts and a current of 5 milliamperes.

The juices and chloroform extracts of lemon and cotton leaves treated with the P^{32} thiol-isomer base and oxalate showed only single spots which migrated distances identical with those of the systemics applied. This information, together with the almost identical residue values in citrus peels obtained by radiotracer assay and cholinesterase assay strongly suggests that the thiol-isomer base is an endolytic systemic and is not metabolized in the plant to more active materials, as has been found with demeton (Fukuto *et al.* 1955).

Systemic Toxicity.—To estimate the LD_{50} to the two-spotted mite, *Tetranychus telarius*, a series of mature cotton leaves were set with their petioles in solutions of P^{32}-labeled thiol-isomer oxalate. At intervals, the leaves were removed and divided longitudinally. One half of each leaf was used for radioassay and the other half was placed in a Munger cell to determine its 24-hour toxicity to the mites. The concentration vs. mortality data obtained were plotted on log. probit paper and a straight line fitted by eye. The estimated 24-hour LC_{50} for the adult two-spotted mite was 11γ per g. of leaf tissue for the thiol-isomer base, equivalent to 14.6γ per gram of thiol-isomer oxalate.

SUMMARY AND CONCLUSIONS.—The systemic insecticidal behavior of the thiono- and thiol-isomers of *O,O*-diethyl *O*-2(diethylamino)ethyl phosphorothioate was evaluated in cotton and lemon plants by using P^{32} radiotracers. Comparisons were made between the rates of uptake and translocation of the thiol-isomer base and its oxalate salt after applications in solutions to the roots and after topical applications to the stems. When applications were made to the roots, the two materials accumulated in the leaves at about equal rates. When applications were made to the stem, the thiol-isomer base accumulated about 10 times as fast as its oxalate salt, thus showing the influence of lipoid solubility on penetration. In comparative studies the demeton thiol isomer penetrated and accumulated about 3.2 times as rapidly as the thiol-isomer base. The rates of penetration of various salts of the thiol-isomer base were also influenced by solubility factors and varied over a factor of nearly 100 times.

Similar results were obtained in studies of the rates of penetration of these compounds into lemon leaves. The thiol-isomer base penetrated considerably more rapidly than any of its salts.

The amounts and distribution of the thiol-isomer oxalate in orange and lemon fruits was measured after full-coverage spray applications at 40 g. per 100 gallons. No detectable amounts were found in the pulps of either fruit, and the amounts in the peels ranged from 0.08 to 0.32 p.p.m. up to 28 days after application. Certain peel and surface residues were also determined by cholinesterase assay, and the values were in excellent agreement with those from radioassay.

The thiol-isomer base and oxalate were compared with demeton thiol-isomer as cotton-seed treatments. The base accumulated in the cotyledons and true leaves 5 to 10 times as rapidly as the oxalate, and demeton thiol isomer accumulated more rapidly than the thiol-isomer base.

The estimated 24-hour median lethal concentration to the two-spotted spider mite, *Tetranychus telarius* (L.), for the thiol-isomer base in cotton leaves was 11 p.p.m.

No evidence of the metabolism of thiol-isomer base or thiol-isomer oxalate to other toxicants could be detected by paper chromatography or paper electrophoresis of chloroform extracts or juices of lemon or cotton leaves. Thus it appears that these compounds are endolytic systemic toxicants.

REFERENCES CITED

Diggle, W. M., and J. C. Gage. 1955. The determination of R-6199 residues in plant tissues. I Development of a method. Tech. Rept. Imperial Chemical Industries, Dec. 1955.

Fukuto, T. R., and R. L. Metcalf. 1954. Isomerization of β-ethyl mercaptoethyl diethyl thionophosphate (Systox). Jour. Amer. Chem. Soc. **76**: 5103–6.

Fukuto, T. R., R. L. Metcalf, R. B. March, and Marion G. Maxon. 1955. Chemical behavior of systox isomers in biological systems. Jour. Econ. Ent. **48**: 347–54.

Fukuto, T. R., and E. M. Stafford. 1956. The rearrangement of *O,O*-diethyl *O*-2-diethylaminophosphorothionate. Ms. in preparation.

Ghosh, R., and J. F. Newman. 1955. A new group of organophosphorus insecticides. Chem. and Indus. 1955: 118

Metcalf, R. L., T. R. Fukuto, R. B. March, and E. M. Stafford. 1956. The systemic behavior of Systox thiol isomer sulfoxide and methosulfate in plants. Jour. Econ. Ent. **49**(6): 738–41.

Metcalf, R. L., and R. B. March. 1952. Behavior of octamethyl pyrophosphoramide in citrus plants. Jour. Econ. Ent. **45**: 988–97.

Metcalf, R. L., R. B. March, T. R. Fukuto, and Marion Maxon. 1954. The behavior of systox-isomers in bean and citrus plants. Jour. Econ. Ent. **47**: 1045–55.

[2] Dow-Corning Corporation.

The Annual Cycle of Pollen Storage by Honey Bees

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In a quantitative consideration of the honey bee colony it seems that, apart from the presence or absence of the queen, the four most important constituents are bees, brood, honey (or other carbohydrate stores) and pollen. Each of these four shows a very significant annual fluctuation in quantity, the understanding of which is of importance to the study of colony development. Indeed, the only other two major constituents which suggest themselves are the wax combs which, though they are increased from time to time, do not in the same sense fluctuate in quantity, and the store of surplus water. The latter, carried sometimes by the bees themselves and sometimes in unripened honey, may at times be important but has up to the present seemed a less fundamental matter for quantitative study.

The numbers of bees have been measured by Farrar (1931), while more recently Jeffree (1955) has given curves showing the numbers throughout the year. The amount of brood present during the summer has been described by Nolan (1925); and Jeffree (1956) has recorded the quantities present in the winter months. Mitchener (1947, 1955), and others, from records of hives on scales, have given information on the amount of honey stored in each summer month, and Jeffree (1956) has already reported on the quantities of pollen within the hive during the winter. However, despite the above measurements of bees, brood and stores, and the considerable amount of work which has already been done on the types and amounts of pollen coming into the hive in the summer as determined by the use of pollen traps (Todd & Bishop 1940, 1941; Synge 1947), there has been no comparable record of the surplus amounts found stored in the combs in the summer. The quantities brought in by the foragers from day to day are obviously not the same as the quantities remaining as pollen stores, since the rate of utilisation may at times be less and at other times may exceed the rate as which pollen is coming in.

It is now possible, however, to present the results of 430 estimations made in this Department between the years 1945 and 1953 showing the amounts of pollen stores found in the combs in different months throughout the year. The measurements were made by means of a grid graduated in square inches, all bees having been first shaken off the combs. An average of 25 healthy, queen-right colonies were studied each year and the mean amounts of pollen are given in figure 1, together with the number of measurements making up each mean value. (During the winter months changes within the hive occur relatively slowly and for this reason the figures for October and November, December and January, and February and March have been grouped together.)

As can be seen from figure 1, pollen is present in the hive throughout the year but only in very small amounts during the winter. A sudden rise occurs from April onwards and the peak is reached in June, July and August, after which there is a rapid fall again to the winter level. The shape of this curve is somewhat similar to that

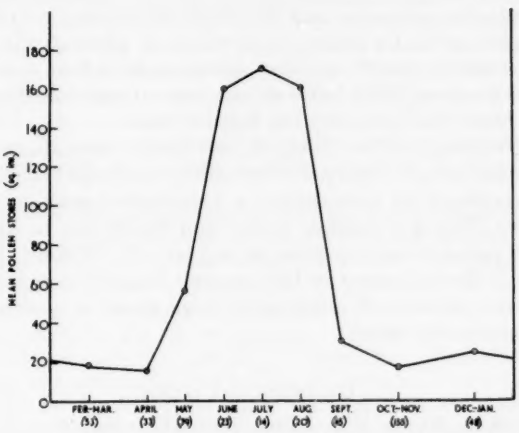

Fig. 1.—Mean quantities of stored pollen (figures in brackets indicate number of estimations).

of the brood curve but has a later rise, which would indicate that the spring increase in brood-rearing is not entirely dependent on the quantity of pollen stored in the hive.

It is probable that as pollen is brought into the hive during the spring most of it is almost immediately used by the bees, while the amount stored represents the excess of intake over consumption. In mid-summer this excess becomes considerable. Towards autumn the bees are consuming pollen faster than they store it, and the latter stages of the decline and final rather abrupt termination of the main brood-rearing cycle correspond closely with the rapid fall to a very low level in the amount of stored or reserve pollen.

Approximately 7½ square inches of pollen represent 1 ounce (Todd & Bishop 1941), and the peak value of 171 square inches in July is therefore the equivalent of 23 ounces. This figure is for normal and rather small colonies (average peak strength about 24,000 bees) in a not very rich district near Aberdeen, and although it could perhaps be higher in other districts the fact of an important summer peak as shown here is likely to be general in temperate regions.

Allen & Jeffree (1956), from measurements of stored pollen in colonies of various sizes, found that colony size influences the amount of pollen stored. The regression coefficients of pollen (measured in $\sqrt[3]{\text{sq. ins.}}$) on colony size (measured in thousands of bees) showed no evidence of systematic fluctuations throughout the year, and a mean regression coefficient calculated on the data for all months together thus gave the best estimate of the value for any individual month. This mean value was 0.0826 ± 0.009. It would be expected, therefore, that at the peak of the pollen curve in July a colony of 34,000 bees would have approximately 260 sq. ins. of stored pollen, and a colony of 40,000 bees approximately 325 sq. ins. (43 ounces).

Observations in this Department have indicated that the state of affairs in queenless colonies is considerably different from that shown in figure 1. In such colonies there are larger quantities of pollen, especially in the autumn and winter, due to the lack of brood. Thus, abnormal amounts of pollen in the combs particularly from September onwards, may be a valuable indication to the beekeeper that a colony is queenless, or alternatively, if queenright, that it has been queenless for a time during late summer. In the latter circumstance it may sometimes be found that the queen has failed to mate.

SUMMARY.—Four hundred and thirty estimations of the amount of stored pollen in healthy queenright colonies throughout the year showed a well-marked peak during June, July and August, while from September to April the pollen stores remained at a relatively constant, low level. Queenlessness in late summer frequently resulted in the presence of abnormally large stores of pollen in autumn and winter.

REFERENCES CITED

Allen, M. D., and E. P. Jeffree. **1956.** The influence of stored pollen and of colony size on the brood-rearing of honeybees. Ann. Appl. Biol. **44:** (4): 649–56.

Farrar, C. L. 1931. A measure of some factors influencing the development of the honeybee colony. Massachusetts State Coll. Library, Amherst, Mass. 185 pp. (Typewritten).

Jeffree, E. P. 1955. Observations on the decline and growth of honey bee colonies. Jour. Econ. Ent. **48:** 723–6.

Jeffree, E. P. 1956. Winter brood and pollen in honey bee colonies. Insectes Sociaux **3**(3): 417–22.

Mitchener, A. V. 1947. Manitoba honey flows 1924–1946. Jour. Econ. Ent. **40**(6): 854–60.

Mitchener, A. V. 1955, Manitoba nectar flows 1924–1954, with particular reference to 1947–1954. Jour. Econ. Ent. **48**(5): 514–8.

Nolan, W. J. 1925. The brood-rearing cycle of the honey bee. Bull. U. S. Dept. Agric. **1349:** 1–56.

Synge, A. D. 1947. Pollen collection by honey bees. (*Apis mellifera*). Jour. Anim. Ecol. **16:** 122–38.

Todd, F. E., and R. K. Bishop. **1940.** Trapping honey bee-gathered pollen and factors affecting yields. Jour. Econ. Ent. **33:** 866–70.

Todd, F. E., and R. K. Bishop. **1941.** The role of pollen in the economy of the hive. Circ. U. S. Bur. Ent. No. E-536: 1–9.

The Importance of *Mesoleius tenthredinis* Mor., A Parasite of the Larch Sawfly, in New York State[1]

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Between 1909 and 1956 some 36 million European and Japanese larch, *Larix decidua* Mill. and *L. leptolepis* (Sieb. and Zucc.) Gord., were planted on reforestation areas of New York State. Both of these larch species are susceptible to attack by the larch sawfly, *Pristiphora erichsonii* (Htg.).

At one time *Mesoleius tenthredinis* had been an important parasite of the sawfly, but recently the host developed a strong immunity to the parasite in the Canadian Prairie Provinces (Muldrew 1953, Lejeune & Hildahl 1954). Drooz (1953) noted the immunity factor in Minnesota larch sawfly larvae. He also noted immunity in Wisconsin specimens.[3] Parasitism now rarely amounts to 5% in these areas.

It was deemed important to learn the status of the parasite in areas outside of the known larch sawfly outbreak limits. In 1956 larch sawfly activity was observed in a small plantation of 25-foot Japanese larch near Coventry, Chenango County, New York (Collins & Yops 1956). Sawfly cocoons were collected by W. E. Smith, Forest Pest Control Foreman, in August 1956, and forwarded to the writer for examination. Parasitism by *Mesoleius tenthredinis* amounted to 56% of the 95 live larvae in the collection. There were no other parasites present.

Four "nodules" which were suspected of containing encapsulated parasite eggs were negative. Two of these were sent to Muldrew for confirmation. He reported (1956) that no trace of egg chorions was found within the capsules.

According to these observations the immunity reaction is not present in the New York sawfly, and *Mesoleius tenthredinis* remains an important parasite of the larch sawfly in that area.

REFERENCES CITED

Collins, D. L., and C. J. Yops. **1956.** Personal communications.

Drooz, A. T. 1953. Larch sawfly investigations in Minnesota, 1952. Jour. Econ. Ent. **46**(5): 826–8.

Lejeune, R. R., and V. Hildahl. **1954.** A survey of parasites of the larch sawfly (*Pristiphora erichsonii* (Hartig)) in Manitoba and Saskatchewan. Canadian Ent. **86**(8): 337–45.

Muldrew, J. A. 1953. The natural immunity of the larch sawfly (*Pristiphora erichsonii* (Htg.)) to the introduced parasite *Mesoleius tenthredinis* Morley, in Manitoba and Saskatchewan. Canadian Jour. Zool. **31:** 313–32, illus.

Muldrew, J. A. 1956. Personal communication.

[1] Accepted for publication December 10, 1956.

[2] Entomologist stationed at the Lower Peninsula Forest Research Center, East Lansing, Michigan. The Center is maintained in cooperation with Michigan State University.

[3] Unpublished material, Lake States Forest Experiment Station.

SCIENTIFIC NOTES

Two Practical Methods of Identifying Types of Ambrosia Beetle Damage[1]

J. M. KINGHORN[2]

It is often difficult to obtain adults of wood inhabiting beetles for identifying species. Since the damage and habits of the two most common ambrosia beetles in British Columbia differ considerably, it is necessary to distinguish between the two whenever records are taken. Adults of these species, *Trypodendron lineatum* (Oliv.) and *Gnathotrichus sulcatus* (Lec.) can be separated easily, because the former are relatively short and stout in comparison with the latter. Since the progeny of both develop in larval niches cut into the end grain of the wood, the damage of each can also be identified by the proportion of length to width of mature larval or pupal niches. However, two methods, based on size of entrance holes and boring dust, obviate the necessity of cutting into the wood.

The entrance holes of *Trypodendron* are slightly larger than those of *Gnathotrichus*. Inexperienced workers usually find it difficult to judge consistently this difference in hole diameter. By carefully measuring a series of entrance holes made by both species, it was found that a simple gauge can be used to ensure accurate determinations.

Measures of 100 holes of each species gave mean values of 1.68 mm. (0.066 inch) and 1.30 mm. (0.052 inch) for *Trypodendron* and *Gnathotrichus*, respectively. No overlapping was encountered, and as expected, a statistical test showed a highly significant difference between the averages. Fifteen holes of each species were measured in wood when it was saturated and again when oven dry. The change in hole size caused by swelling of the wood was insufficient to cause any overlapping in the hole diameters of the species. The shank of a No. 53 wire gauge twist drill was chosen for the test gauge. The diameter of this drill (0.0595 inch) is small enough to fit into the smallest *Trypodendron* holes, but is too large to fit the largest *Gnathotrichus* holes. Failure to fit the gauge into a hole indicates a *Gnathotrichus* attack, while the converse indicates attack by *Trypodendron* (Fig. 1A).

Even under gross examination, the white boring dust extruded from the egg galleries while the tunnels are being extended, but before larval frass is pushed out, differs sufficiently to distinguish between these species. The piles of *Trypodendron* dust are agglomerate, the irregular-sized particles tending to cling together even when dry (Fig. 1B), whereas those of *Gnathotrichus* are made up of loose individual particles of uniform size. When a single layer of *Gnathotrichus* dust is sifted on the palm of one's hand, it can be seen that the particles are all uniform and comma-shaped (Fig. 1C). In third dimension, each particle resembles a miniature spoonful scooped from a melon. Although a few comma-shaped chips can be seen in *Trypodendron* boring dust, most particles are made up of torn fragments of wood that are not uniform in size or shape.

In other regions where many ambrosia species and genera are common, these simple methods of identification may not be practical, but it is possible that even among heterogeneous beetle populations, specific boring dust characteristics will help to identify species. For example, among other British Columbia ambrosia beetles, it is known that at least some extrusions of *Platypus wilsoni* Sw. are distinct, splinter-like fragments. Also, a difference between boring dust of beetles of the same size and genus has been noted. *Gnathotrichus alni* Blkm. extrudes comma-shaped particles, but their size is distinctly smaller than those of *G. sulcatus*.

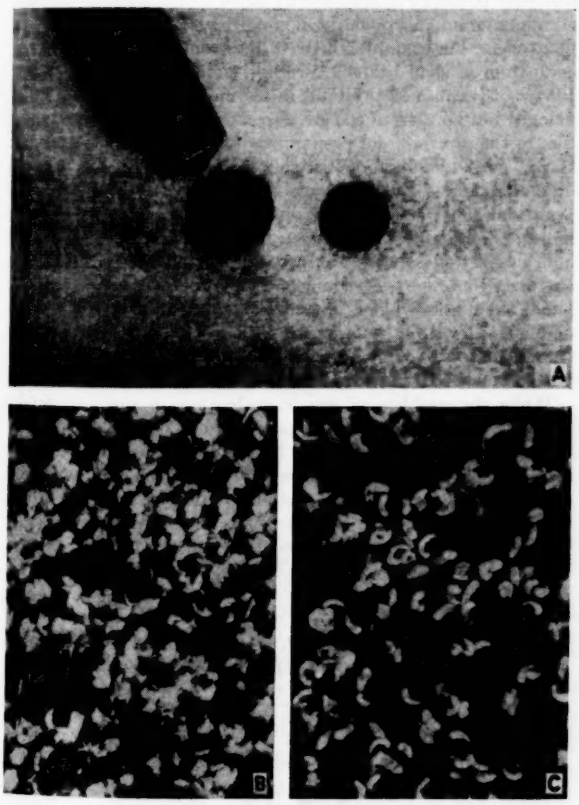

FIG. 1. (A) The size of the test gauge in comparison to the entrance holes of *Trypodendron* (left) and *Gnathotrichus* (right). (B) Particles of boring extrusions of *Trypodendron* and (C) *Gnathotrichus*. Approx. 7×.

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Progress Report on a Regional Approach to the Problem of Flavor Evaluation of Fruits and Vegetables as Influenced by Pesticides[1]

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The Directors of the Northeast Region Agricultural Experiment Stations allocated funds in 1954 for a regional project to determine the effect of the use of pesticides on the flavor of fruits and vegetables. Funds became available and work started in July of that year. During the first year food technologists and entomologists from the Connecticut, Maine, Maryland, Massachusetts, New Jersey, and Delaware stations participated; in 1955 New York (Cornell) and Pennsylvania joined the cooperative effort. The Entomology Research Branch of the U. S. Department of Agriculture is also interested in and contributing to this project.

[1] Scientific Article No. A591. Contribution No. 2755 of the Maryland Agricultural Experiment Station, Departments of Entomology and Horticulture, University of Maryland, College Park.

[2] L. P. Ditman, Research Professor, Department of Entomology; A. Kramer, Professor of Horticulture, University of Maryland.

The first objective was to determine a comparatively simple, reliable, and uniform taste panel method or procedure for evaluating flavor of processed fruits and vegetables. Sufficient progress was made on this phase of the problem in 1954 and 1955 to enlarge the scope of the work to include screening of vegetables and fruits treated with new pesticides and to make some observations on the effect of cultural practices as influencing flavors caused by pesticides. The New Jersey station has devoted its major effort to physicochemical means of detecting factors responsible for or associated with flavor differences. Connecticut and Massachusetts are also contributing to this phase of the work.

THE 1954 WORK.—It was decided by the cooperators that a multiple comparison type of test would be a better method of evaluating flavors as influenced by pesticides than a triangular or paired comparison procedure since in the multiple comparison test actual numerical values are assigned to varying degrees of flavor difference while the other tests merely express a preference for one sample over another. Thus results with the multiple comparison method were found to provide the proper answers with a minimum sized panel and a minimum number of tastings. The results of the multiple comparison test, in which the product with different treatments is presented to the panel a number of times just as one replicates randomized blocks of treatments in the field, lend themselves readily to statistical treatment. Results of such tests by different panels should be reproducible. Also, the scale of values used in the multiple comparison test may be spread to include a range of product flavors from unacceptably poor to superior (Kramer & Ditman 1956).

In 1954 apples grown in Connecticut and processed by the Massachusetts station, squash grown and processed by the Massachusetts station, and snap beans grown and processed by the Maryland station, were evaluated flavorwise by the Maine, Massachusetts, and Maryland stations. Results of the three stations on squash and apples were in reasonable agreement; there was no significant difference in flavor of apple sauce made from apples receiving several different spray schedules, while squash receiving one of three insecticide-fungicide treatment schedules was found inferior to other treatments at all three stations; results on beans were not in agreement for reasons mentioned later. These results, while only of a preliminary nature, were of considerable value in planning the 1955–56 season's work both from the standpoint of the food technologists and of the entomologists concerned.

THE 1955–56 WORK.—This was largely a repetition of the previous year's effort but with added refinements gained from the previous experience. The same vegetables and fruits were grown and processed by the same stations but were evaluated by the Pennsylvania and New York (Cornell) stations also. The most important result of the 1955–56 work was closer agreement of evaluation of all products at all participating stations. Wiley *et al.* (1956) have summarized the results of the 2 years' work and it is not the intention of the authors to go into detail on methods at this time. The main points of interest are that the multiple comparison, or variables, method of evaluating flavors of samples of fruits and vegetables treated with pesticides gave reasonably uniform results. The greater the flavor differences, the closer the agreement between panels; the less the flavor differences of a set of samples, the greater the variation of results between panels. It was found that there was little difference in the results obtained by a comparatively large (35–40) untrained panel and a small (5–7) trained panel of judges. The smaller trained panel tended to assign a greater range of differences between samples, and in some cases, required a greater number of tastings to give highly significant (1% level) results. They also found that a balanced factorial experiment, that can be utilized with the multiple comparison method, greatly facilitated the combining of data from the various laboratories for statistical treatment.

DISCUSSION.—In general, the participants feel that a regional approach by cooperating food technologists and entomologists to evaluate flavors of fruits and vegetables resulting from the use of pesticides is an extremely important step toward a valid solution of the problem. Food technologists tend to emphasize, and rightly so, the problems of methodology while the entomologists who are responsible for insecticide recommendations are primarily concerned with the practical application of results. Working together they get a broader insight into the problems concerned, which in the end should result in more comprehensive and practical conclusions.

The multiple comparison or variables method appears to be well suited for the evaluation of product flavors as influenced by pesticides since definite values are assigned varying degrees of flavor difference both inferior and superior. Entomologists are as much interested in improving flavors through increase in plant vigor brought about by protection from insect injury by insecticides as in preventing off-flavors. From the standpoint of the technologist, valid and reproducible results may be obtained with a minimum number of tastings by a small panel of judges. From the standpoint of the entomologist this method has only one weakness: panel members, unless thoroughly trained and familiar with products in question, tend to assign the full range of scale values to any set of product being evaluated. This could result in the condemnation of a pesticide which influences flavor very slightly and unimportantly. This weakness will be taken into account and corrected in future work.

Since many entomologists are confronted with the problem of preparing and collecting samples of fruits and vegetables a few precautions may be mentioned. When the primary objective of flavor evaluation is pesticide effect, variation in all other factors that may influence quality and flavor of a product must be kept at a minimum, particularly non-pesticidal factors that affect plant vigor. For example, three stations could not agree on flavor evaluation of snap beans grown and processed in Maryland in 1954, not because of any fault in the methods of the food technologist but because of too great variation in non-pesticidal factors in the growing and preparation of samples. Some plots received considerably more irrigation than others, resulting in differences in vigor of the plants and maturity of the crop. Valid pesticidal effect could not be obtained on such samples. Entomologists also should be extremely careful in harvesting crops and submitting samples for processing, and should avoid old and dirty containers. Touching of the product with contaminated hands should be avoided. Products should be handled promptly and not allowed to deteriorate with age. The results secured from the food technologist will be no better than the samples you give him.

While the problem of flavor evaluations is not completely solved it is felt that definite progress has been made by the regional approach. Some of the basic difficulties appear to have been overcome so that objectives of future work may be broadened. The vast array of new pesticides which must be evaluated alone and in numerous combinations on many crops presents an increased number of problems the prompt solution of which requires an intense cooperative effort.

REFERENCES CITED

Kramer, Amihud, and L. P. Ditman. 1956. A simplified variable taste panel method for detecting flavor changes in vegetables treated with pesticides. Food Technol. X(3): 155–59.

Wiley, Robert P., Alice M. Briant, Irving S. Fagersen, Jean E. Henderson and Elizabeth F. Murphy. 1956. Evaluation of flavor changes due to pesticides—a regional approach. Food Res. (in press).

A Diet for Mexican Fruit Flies[1]

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A diet for adult fruit flies which stimulates and maintains high egg production and viability is essential to satisfy the demands for large numbers of eggs and other stages of the Mexican fruit fly, *Anastrepha ludens* (Loew), required for the investigations carried on at Mexico City in cooperation with the Mexican Government. Such a diet has been found to consist of granulated sugar, orange-juice crystals, and an enzymatic protein hydrolysate of yeast[2] found by Hagen (1953) to contain the necessary nutrients for good reproduction by fruit flies. The substances are given in a dry form. To prevent the orange-juice crystals from deliquescing excessively, the sugar is mixed in at a ratio of 2:1. The yeast hydrolysate is presented to the flies separately, and under conditions of high humidity must be changed frequently.

The highest oviposition previously reported (McPhail & Bliss, 1933, p. 18) was 401 eggs from one female. In order to test the effectiveness of this diet, two oviposition experiments involving 80 pairs of fruit flies were conducted, one in 1954 and another in 1955. The flies were held at a mean temperature of 77° F. (25° C.) and a relative humidity of about 50%.

Table 1 presents data on the females that deposited the greatest number of eggs and also on the viability of the eggs laid by six of the flies during the latter part of their oviposition period. Table 2 contains information regarding oviposition of the 80 paired females in both years. From these data it is evident that, when Mexican fruit flies are given a favorable diet, their egg-laying capacity is much higher than has previously been realized. Such a favorable diet is probably sometimes available to them in the form of aphid honeydew or plant secretions. It is also evident that a high percentage of the eggs laid, at least among the first two-thirds of the total, are viable.

Table 1.—Individual oviposition records of *Anastrepha ludens*.

Female No.	Oviposition Period (Days)	Number of Eggs Laid	Group of Eggs Sampled[a]	Per Cent Viability
	Test begun October 16, 1954			
5	127	4164	—	—
22	113	4119	Nos. 2991–3037	91
	Test begun August 29, 1955			
8	120	3562	Nos. 2499–2513	50
			3429–3453	0
22	71	3232	—	—
33	120	3063	Nos. 2346–2387	85
34	127	3009	—	—
35	85	3433	Nos. 2374–2444	93
38	119	3300	Nos. 3114–3139	92

[a] Numbers indicate the time of sampling in relation to the total number of eggs laid.

Table 2.—Summary of oviposition records of 80 paired females of *Anastrepha ludens*.

Year	Days Required for Pre-Oviposition Period		Duration of Oviposition Period (Days)	Total Number of Eggs Per Female
	Fewest	Average		
1954	11	18	87	1,056
1955	9	16	73	1,769

References Cited

Hagen, Kenneth S. 1953. Influence of adult nutrition upon reproduction of three fruit fly species. Third Special Report on the Oriental Fruit Fly, Exhibit C, pp. 72–76. Published by Senate of State of California.

McPhail, M., and C. I. Bliss. 1933. Observations on the Mexican Fruit Fly and some related species in Cuernavaca, Mexico, in 1928 and 1929. U. S. Dept. Agric. Circ. **255**, 24 pp., illus.

[1] Accepted for publication November 5, 1956.

[2] MRT, obtained from Marvin R. Thompson, Inc., Stamford, Conn.

Alfalfa Weevil Control in Delaware, 1955, with Observations on Pea Aphid and Meadow Spittlebug[1]

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Beginning in 1953, the year after its discovery in Delaware, efforts have been directed toward control of the alfalfa weevil, *Hypera postica* (Gyll.). Milliron & MacCreary (1955) reported on the character and extent of injury, seasonal development, population trends and control for the years 1953 and 1954; and Milliron (1956) has given an account of its activities in 1955.

TESTS.—In 1955, one-half acre plots (three replicates per treatment) were established on the University Farm, Newark, in a four-year-old alfalfa planting. The insecticides, dieldrin (1½ pints; 4 oz. actual), heptachlor (1 pint; 4 oz. actual) and endrin (1 pint; 3.2 oz. actual), were applied April 13, with a low-pressure, low-gallonage sprayer in 25 gallons of water per acre. Foggy weather prevailed during the late afternoon of that date, followed by rain (0.67 inch) during the night and early morning of April 14. The mean temperature on April 13 was 45.5° F.

A total of six counts were made, beginning April 15 and ending May 20. Fifteen sweeps per plot were made at each count, and the numbers of alfalfa-weevil larvae and adults and pea aphids recorded. Analysis of variance was calculated for number of larvae and adults present, considering each separately.

The collection data for the different treatments are presented in table 1. A tremendous increase in the number of larvae occurred between the 21-day count (May 4) and the 29-day count (May 12). The unsprayed check showed a 20-fold increase. In the sprayed plots, the increase was even greater; however, the totals did not approximate that recorded for the check. The following is a tabulation of the cumulative effect of the toxicants on the 21st day (May 4) and 29th day (May 12) after treatment:

	21-Day Totals			*29-Day Totals*		
Toxicant	*Larvae*	*Adults*	*Per Cent Control*	*Larvae*	*Adults*	*Per Cent Control*
Dieldrin	4	31	92	391	70	78
Heptachlor	9	46	87	879	80	54
Endrin	16	273	33	606	318	56
Check	134	298	—	1748	336	—

The per cent control was computed on the basis of larvae and adults together, as a direct comparison with counts on the untreated check. Endrin made a poor showing at 21 days, owing to its failure to control adults, although it remained effective against larvae. There was a definite lessening of the protection at 29 days, but the accumulation of larvae in the check plots

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Table 1.—Results of insecticide tests against the alfalfa weevil on first crop alfalfa at Newark, Delaware, April 13 to May 20, 1956.

	Average Number Collected and Average Per Cent Control on Dates Indicated after Treatment[a]																	
	2 Days (April 15)			9 Days (April 22)			17 Days (April 30)			21 Days (May 4)			29 Days (May 12)			37 Days (May 20)		
Toxicant[b]	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C
Dieldrin	0.6	0	96	0	1.3	96	0.6	2.0	94	0	7.0	86	129.0	13.0	74	950.0	7.3	59
Heptachlor	0	0.6	96	0	1.3	96	1.3	2.0	92	1.6	11.3	74	290.0	11.3	45	2300.0	6.3	0.2
Endrin	1	6.6	49	0	30.3	16	0	25.3	41	4.3	28.3	34	196.6	15.0	62	1173.0	5.6	50
Check	2	12.0		1.6	27.3		16.6	28.3		30.3	29.6		586.3	15.0		2433.3	7.3	
L.S.D. at 5% level				ns	20.23		ns			22.16	ns		243.8	ns				
at 1% level								20.35										

[a] Per cent control computed by Abbott's formula (Jour. Econ. Ent. 18: 265–7, 1925), and is based on the sum of larvae and adults. Figures for larvae and adults are the averages of three replicates. A, larvae; B, adults; C, per cent control.
[b] Applied April 13.

was proportionately greater, so that their numbers were significantly higher than in all treated areas. By May 20 (37 days after treatment) it was evident that there was insufficient insecticide remaining on the plants to kill larvae hatching during this period. Adult control was not significant by 21 days after treatment. Dieldrin had somewhat greater residual effect than the other two materials at the 29- and 37-day counts.

On May 20, the date of the last count, more than 7000 larvae were taken in 45 sweeps from the unsprayed check. The dieldrin- and endrin-sprayed plots had substantially fewer larvae; however, considerable damage was noticeable on all plots. The quality of the hay decreased rapidly; and, on the cutting date (May 26), severe injury had resulted regardless of the insecticide used.

The stubble was not treated. By mid-June, the second growth on the unsprayed check was in striking contrast to that on the plots sprayed on April 13. On June 17, a 12- to 14-inch growth was present on the treated areas, while no growth was evident on the untreated one. A few days later, following a heavy rain, new shoots began to appear on the untreated check. By July 7, recovery was such that there was only a 3- to 4-inch differential between it and the treated plots, although bloom on the check was much delayed on that date. On July 11, samples of hay were cut from 100-square-foot areas in all plots, air-dried and weighed. The yield from the check totaled 2516 pounds per acre, while the average for all treated plots was 3039 pounds per acre (maximum difference between any two treated plots did not exceed 145 pounds per acre).

This difference of $\frac{1}{4}$ ton per acre in the yield for the second cutting appears to be a factor that, normally, is not considered. Actually, had it not been for the rains in late June, it is doubtful if the check would have recovered to such an extent. From these data, it is apparent that damage resulting from weevil-feeding on the first cutting, particularly if the feeding is extremely heavy, markedly affects the yield of the second growth. This possibility should not be overlooked in any decision concerning the need for controlling the alfalfa weevil. There has been a feeling among farmers that, even if the first cutting is badly damaged, the second cutting will provide a normal crop of hay.

Counts of the pea aphid, *Macrosiphum pisi* (Harr.), were made simultaneously with those for alfalfa weevil, except that the last count (May 20) was omitted. Neither dieldrin nor heptachlor provided any control of this insect. However, endrin greatly reduced the aphid population, which was 16 times greater in the check than in the plot treated only once with endrin.

On May 19, counts were made of the number of spittle masses of the meadow spittlebug, *Philaenus leucophthalmus* (L.), on 10 square yards in each plot. A yard-square frame was used to delimit the areas counted. A very low infestation was recorded. The unsprayed check averaged 4.9 masses per square yard, while the averages for the treated plots were as follows: dieldrin, 0.07; heptachlor, 1.2; and endrin, 0.03.

References Cited

Milliron, H. E., and Donald MacCreary. 1955. The Alfalfa Weevil in Delaware, 1953–54. Jour. Econ. Ent. 48: 283–9.

Milliron, H. E. 1956. Observations on the Alfalfa Weevil in Delaware, 1955. Jour. Econ. Ent. 49: 441–3.

Alfalfa Weevil Control with Granulated Insecticides in Virginia

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In recent years the alfalfa weevil, *Hypera postica* (Gyll.), has become one of the most important forage pests in the East, and its occurrence and early spread has been adequately covered in previous papers (Poos & Bissell 1953, App 1954, Milliron & MacCreary 1955). The spread in Virginia has been especially rapid, as the infestation has increased from two counties in 1952 to 70 counties in 1956. The damage which is caused mostly by larval feeding has been serious on the first cutting, often causing a total loss, and the second growth is often seriously retarded. In heavily infested fields where control measures are not applied the stand may be lost in a single season.

Control studies conducted in Virginia during 1955 and 1956 indicated that several of the chlorinated hydrocarbon insecticides applied as low-pressure, low-gallonage sprays gave excellent control of the alfalfa weevil (Muka 1956). Other workers (App 1954, Milliron & MacCreary, 1955) have also indicated success with low-pressure sprays. Previous work (App 1954) has shown that proper timing in the spring is critical in obtaining protection with one application. In some seasons cool wet weather prolongs the egg-laying period of the weevil so that more than one application of spray may be required for protection of the crop.

In an effort to explore the possibility of a single insecticidal application which would not require critical timing, the writer investigated the use of granulated insecticides applied in the dormant period of plant growth. The experiment involved a single application to each plot in December or March, and the results obtained are reported here.

A Gandy[3] manually-operated fertilizer spreader of commercial design was employed to distribute the materials used in this experiment. In an effort to reduce variations in the amount of granulated material applied to each of the four replicates, a low-recording speedometer was installed on the fertilizer spreader. The operator found that it was possible to apply very uniform

[1] Now in the Department of Entomology, Cornell University.
[2] The assistance of Mr. W. A. Carter in this work is acknowledged.
[3] E. S. Gandrud Company, Inc., Model 3–50.

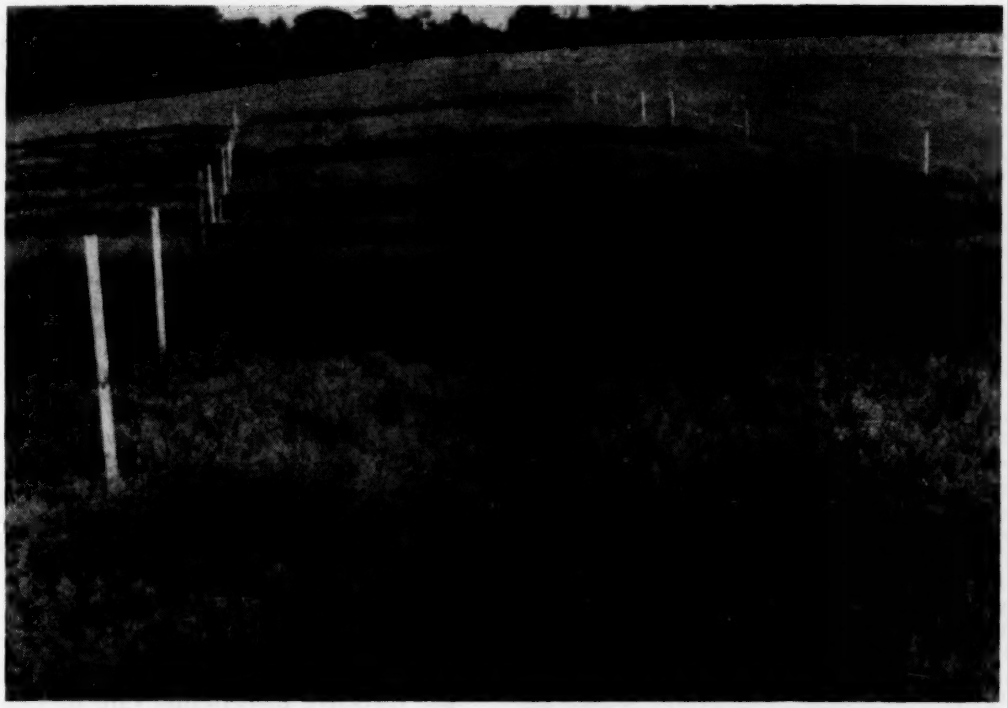

FIG. 1.—Alfalfa field showing treated and untreated plots for control of alfalfa weevil with granulated insecticides.

amounts of granulated material with this spreader when operated at a speed of 2 miles per hour.

The plots were 1/100 acre (12 ft.×36.3 ft.) in size. Each treatment was replicated four times and randomized in blocks in a split plot design. Each plot was separated from the next adjacent plot by an untreated area of the same size. On December 29, 1955, one-half of the plots were treated while the alfalfa was completely dormant. On March 30, 1956, just as the plants were starting spring growth, the remainder of the plots were treated.

Table 1.—Effectiveness of granulated insecticides against the alfalfa weevil on alfalfa. Goochland County, Virginia. 1956.

MATERIAL	PER CENT ACTUAL TOXICANT	POUNDS TOXICANT PER A.	TOTAL GRAMS PER 1/100 A.	NUMBER LARVAE IN 25 SWEEPS OF NET[a]	PER CENT REDUCTION OF LARVAE OVER UNTREATED CHECK
Treated December 29, 1955					
Aldrin	5.0	1.5	136.2	361	13.9
Dieldrin	5.0	1.0	90.8	237	43.4
Parathion	2.5	2.5	908.0	287	31.5
Lindane	1.0	2.0	454.0	279	33.4
Heptachlor	2.5	1.5	272.4	61	85.4
Untreated	—	—	—	419	—
Treated March 30, 1956					
Aldrin	5.0	1.5	136.2	140	66.6
Dieldrin	5.0	1.0	90.8	203	51.5
Parathion	2.5	2.5	908.0	629	—
Lindane	1.0	2.0	454.0	193	53.9
Heptachlor	2.5	1.5	272.4	5	98.8
Untreated	—	—	—	419	—

[a] Made on May 11, 1956. Average of insect counts made in four replicates.

The insecticides used were proprietary granulated formulations (on clay-type carrier) of aldrin, dieldrin, parathion, lindane and heptachlor which were stated to be of a 30–60-mesh size. On May 11, 1956, the insect population was sampled by taking 25 sweeps in each plot with a 15-inch diameter collecting net. The data obtained are summarized in table 1. The photograph (fig. 1) indicates the condition of the treated and untreated plots on May 11, 1956.

The data show that the application of these granulated insecticides to alfalfa either in December or March gave poor to excellent control of alfalfa weevil larvae. Of the materials used, heptachlor, applied at the rate of 1½ pounds of actual toxicant per acre, gave the best control. The other toxicants at the dosages used gave some measure of plant protection. The spring application of aldrin, dieldrin, and lindane gave slightly better results than the winter application. The reason heptachlor proved to be so superior in the granulated form in this test is not immediately known because aldrin, dieldrin, lindane and heptachlor applied as sprays are effective materials for alfalfa weevil control (App 1954). Work on Japanese beetle larvae with granulated insecticides indicated that the distribution of a material is in relation to the total bulk applied per acre (Fleming 1956). It will be noted (table 1) that the total bulk of heptachlor applied was double that of aldrin or dieldrin. These results therefore suggest the possibility that both the dosage as well as the total bulk applied are factors related to the degree of control obtained.

SUMMARY.—A preliminary experiment was conducted in central Virginia for control of the alfalfa weevil with five granulated insecticides. These insecticides were applied to separate plots at two different dates during the dormant period. The most effective of these materials was heptachlor which gave excellent control at the rate of 1½ pounds of actual toxicant per acre applied either in the winter or in the spring.

REFERENCES CITED

App, B. A. 1954. Insecticide tests to control the alfalfa weevil

and the meadow spittlebug in Maryland. Jour. Econ. Ent. 47: 313–6.
Fleming, W. E. 1956. Personal correspondence.
Milliron, H. E., and Donald MacCreary. 1955. The alfalfa weevil in Delaware, 1953–54. Jour. Econ. Ent. 48: 283–9.
Muka, A. A. 1956. Unpublished report, Entomology Section, Virginia Agricultural Experiment Station.
Poos, F. W., and T. L. Bissell. 1953. The alfalfa weevil in Maryland. Jour. Econ. Ent. 46: 178–9.

Microsyringe Adapter[1]

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A new microsyringe for use in the artificial insemination of queen honey bees was developed by Mackensen in 1948. Further refinements were made by Mackensen in 1954. Mackensen's syringe differs from previous ones in that the metal plunger does not extend into the syringe tip but functions to stretch a rubber diaphragm into a 60° cone-shape cavity in the base of the tip. Reversal of the movement of the metal plunger permits the stretched diaphragm to resume its original position drawing semen into the tip in the process. It was found necessary to use a liquid plunger in the tip itself, rather than air, because the compressibility of air makes the smooth movement of the viscous semen in the syringe tip impossible. In actual use the tip is filled with water and then enough is ejected to provide space for the semen and to allow an air bubble to form between the semen and the water plunger.

Mackensen's syringe is very satisfactory for the artificial insemination of queen honey bees, and presumably would also be satisfactory for other micro-injection purposes where the syringe tip need be no smaller than the standard one furnished with the syringe.

When the writers had occasion to use considerably smaller injection tips it became necessary to design an adapter for the Mackensen syringe to receive the smaller tip. This adapter, shown in figure 1, is made of 11/32″ diameter Lucite and is 1¼″ long when assembled. It consists of three parts: a base (A), a seating tip (B), and a rubber gasket (C). One end of the base is reduced in diameter and is threaded to fit into the socket of the metal syringe barrel. It is also provided with a 60° cone-shaped terminal cavity. The other end of the base is bored ⅜″ deep to a flat seat for the rubber gasket, and is threaded to accept the seating tip. The threaded end of the seating tip is flat to provide contact surface for the gasket. The other end is tapered. The center bore is 0.025 inch in diameter and extends throughout the length of the adapter. The gasket is 7/32″ in diameter and 1/16″ thick.

To use the adapter, a small center hole is made in the rubber gasket and the gasket is placed on the gasket seat. The two parts of the adapter, A and B, are then screwed together loosely and the basal end of the glass micro-tip is pushed through the gasket hole. The socket end of the metal syringe barrel is now filled with water and the threaded reduced end of the base of the adapter is screwed into this socket which, together with capillary action, forces water into the glass micro-tip. The two parts of the adapter are then screwed together firmly to press the gasket tightly against the micro-tip and make an effective seal. Enough water must be ejected from the syringe to provided space for the fluids to be taken up and for a small airbubble between the fluids and the water plunger.

The micro-tip can be drawn from 3/16-inch glass tubing by drawing first a capillary tube and then warming the capillary tube high above the flame and pulling the tube with constant tension. When drawn this way the diameter of the bore of the micro-tip may be made as small as 0.0035 to 0.0040 mm. or 0.00014 to 0.00016 inch.

REFERENCES CITED

Mackensen, Otto. 1948. A new syringe for the artificial insemination of queen bees. Amer. Bee Jour. 88(8): 412.
Mackensen, Otto. 1954. Some improvements in method and syringe design in artificial insemination of queen bees. Jour. Econ. Ent. 47(5): 765–8.

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The Use of Chlorinated Polyphenyls to Increase the Effective Insecticidal Life of Lindane[1]

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The tests described in this paper were designed to investigate the possibilities of extending the effective life of lindane by the addition of a chlorinated polyphenyl to the spray solution. Other research workers, in preliminary studies with cockroaches and house flies, have reported some evidence that there was a marked increase in the effective life of lindane when such material was added to the toxicant formulation (Hornstein & Sullivan 1953, Sullivan *et al.* 1955). From their tests they concluded that the effective life of lindane may be increased 10 to 20 times. Therefore, the author attempted to confirm their results under field conditions with an insect of economic importance to the shade tree field.

The materials selected for testing were 25% lindane wettable powder and Aroclor No. 5460, a chlorinated polyphenyl produced exclusively by the Monsanto Chemical Company (Benignus, n. d.). The insect used was the elm leaf beetle, *Galerucella xanthomelaena* (Schr.).

The resinous Aroclor, which is insoluble in water, was dis-

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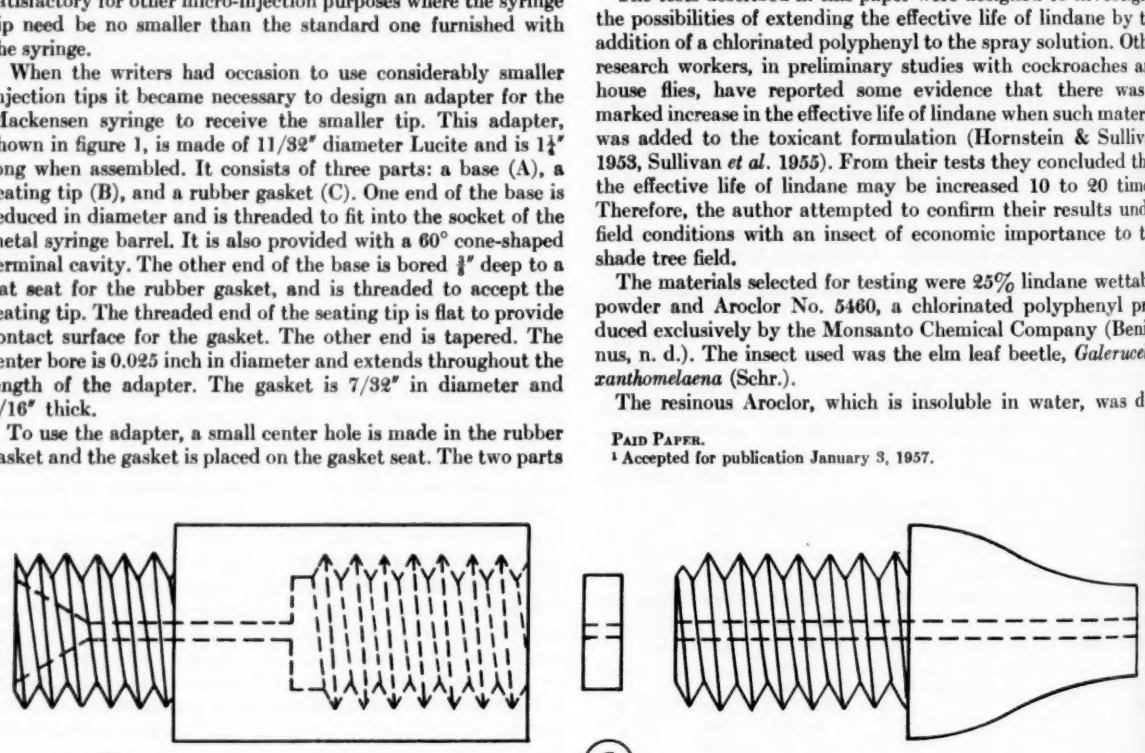

FIG. 1.—Microsyringe adapter disassembled. (A) base, (B) seating tip, (C) rubber gasket.

Table 1.—**Effect of lindane and lindane-Aroclor on the elm leaf beetle.**

Days Treated Prior to Infesting	Per Cent Dead After			
	Lindane		Lindane-Aroclor	
	2 Days	5 Days	2 Days	
	Test 1 (Larvae)			
4	70	100	100	
7	65	100	100	
11	70	100	100	
14	45	80	100	
Untreated	10	25[a]		
	Test 2 (Adults)			
8	5	80	100	
36	0	15	100	
Untreated	0	0[b]		
	Test 3 (Adults)			
	1 Day	*3 Days*	*1 Day*	*3 Days*
23	0	60	60	100
35	0	70	70	100
41	0	90	90	100
58	0	0	0	50
65	10	40	40	70
71	0	10	10	90
Untreated	10	20		

[a] The remainder pupated within 20 days.
[b] None dead after 7 days.

solved in xylene at the rate of 1 pound of Aroclor No. 5460 to 1.25 pints of xylene and 1 fluid ounce of Triton X-100, added as an emulsifier. This mixture was then added, along with 1 pound of 25% lindane wettable powder, to enough water to make 100 gallons. This gave a suspension containing four parts of Aroclor to one part of actual toxicant.

Prior to carrying out the tests with lindane, it was deemed advisable to determine if Aroclor displayed any toxic properties when used alone. To determine this, a potted elm sapling was sprayed with a suspension of lindane, another with a suspension of lindane and Aorclor, a third with an emulsion of Aroclor, and a fourth with ordinary tap water. The plants were then allowed to dry. Leaves from each lot were removed, placed in separate trays and infested with adults of the elm leaf beetle. Twenty adult beetles were used per treatment, observed closely and their ensuing actions were recorded. The beetles which were placed on the lindane-treated leaves and on the lindane-Aroclor-treated leaves were affected almost immediately. After 1 hour all 40 beetles were on their backs showing only a slight movement of the appendages and after 24 hours all were dead. The beetles which were placed on the Aroclor-treated leaves, on the other hand, and on those leaves serving as the check displayed no ill effects at the end of an hour. At the end of 24 hours, however, those on the Aroclor-treated leaves appeared to have very little control of their movements. At the end of 48 hours they were on their backs but still alive, but were dead at the end of 72 hours, whereas, those serving as the check appeared perfectly normal. The results of this test indicated that the Aroclor formulations containing no lindane possessed some insecticidal properties.

On June 12, studies were begun in which small elm saplings growing in pots out-of-doors were sprayed with solutions of lindane or lindane-Aroclor. All applications were made with a 2-gallon D. B. Smith compressed air sprayer. The plants were sprayed to the point of run-off and then placed on a bench out-of-doors where they were subjected to the existing weather conditions during the months of June and July. One group of elms was treated with a suspension of 25% lindane wettable powder at the rate of 1 pound to 100 gallons of water while another group was treated with the same suspension to which Aroclor was added. Individual plants within each group were sprayed on different dates in order to provide material suitable for this study. Sufficient Aroclor was not available to treat a third group with an emulsion containing Aroclor alone. Therefore the results presented here give only a comparison between the respective actions of the lindane and the lindane-Aroclor formulations.

On June 28 leaves were removed from the elms of each group, placed in trays and infested with third- and fourth-instar larvae of the elm leaf beetle. Twenty larvae were placed in each tray, an unreplicated test. The results are given in table 1.

Two series of tests were conducted using the adult stage of the elm leaf beetle. On August 7 and again on August 22 adult beetles were placed on leaves removed from previously treated plants. Twenty adults were placed in each tray and, as in Test 1, they were not replicated but there were three replicates of the control or check. The results are also given in table 1.

Perhaps one of the most striking results in the data obtained is the apparent rapidity with which the lindane-Aroclor formulation acted. In Test 1 all larvae placed on leaves treated with the lindane-Aroclor mixture were dead within 2 days whereas,with lindane alone, 100% mortality was not reached until after 5 days. Results were similar with the adult beetles. Ninety per cent mortality occurred at the end of 24 hours and 100% mortality occurred within 3 days on the leaves treated with the lindane-Aroclor formulation even after 41 days had elapsed between the time of treatment and the placing of the adult beetles on the treated leaves.

In summary, the results presented indicate that an increase occurred in the effective life of lindane when Aroclor was added to the formulation. Aroclor when used alone, exhibited a toxic effect on the elm leaf beetle. When it was used in combination with lindane, mortality occurred earlier than from the use of lindane alone. It is possible that the Aroclor might have a synergistic effect on the lindane when the two insecticides are used in combination. Further investigations are needed to determine this point.

References Cited

Benignus, P. G No date. Lindane-Aroclor Combinations. Monsanto Chemical Co., St. Louis, Missouri.

Hornstein, I., and W. N. Sullivan. 1953. The role of chlorinated polyphenyls in improving lindane residues. Jour. Econ. Ent. 46(6): 937–40.

Sullivan, W. N., I. Hornstein, A. H. Yeomans, and Ching-Hsi Tao. 1955. Improved deposits for controlling insects outdoors. Jour. Econ. Ent. 48(2): 153–5.

Growth and Reproduction of the Pink Bollworm on an Amino Acid Medium[1]

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A previous paper (Vanderzant & Reiser 1956) described a medium promoting optimum growth and reproduction of the pink bollworm, *Pectinophora gossypiella* Saund. This medium, however, contained casein and could not be used to determine the requirements for individual amino acids. This paper describes a medium containing L-amino acids instead of protein, on which optimum growth and reproduction of the pink bollworm were obtained.

Preparation of the Medium.—The composition of the

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Table 1.—Composition of an amino acid diet for the pink bollworm.

Portion Sterilized by Filtration						Portion Sterilized by Autoclaving		
Solution No.[a]	Constituent	Mg. per 100 Ml. of Medium	Solution No.	Constituent	Mg. per 100 Ml. of Medium	Solution No.	Constituent	Mg. per 100 Ml. of Medium
1	L-arginine	311	6	Niacinamide	1.0	11	Corn oil (Mazola)	300
	L-histidine (HCl)	75		Calcium pantothenate	1.0		Tween 80[b]	150
	L-isoleucine	113		Thiamin (HCl)	0.25		Cholesterol	50
	L-leucine	175		Riboflavin	0.50		α-tocopherol	10
	L-lysine (HCl)	113		Pyridoxin (HCl)	0.25			
	L-methionine	75		Folic acid	0.25	12	KI	0.4
	L-phenylalanine	150		Biotin	0.02		$CaCO_3$	120
	L-threonine	100		Vitamin B_{12}	0.002		Water to 50 ml.	
	L-tryptophan	75					Agar 3 grams	
	L-valine	138	7	K_2HPO_4	160			
				NaH_2PO_4	80			
2	L-alanine	150		NaCl	20			
	L-aspartic acid	100						
	L-glutamic acid	125	8	$MgSO_4 \cdot 7H_2O$	100			
	Glycine	150						
	L-proline	50	9	$FeSO_4 \cdot 7H_2O$	20			
	L-serine	50						
3	L-cystine	50	10	$CuSO_4 \cdot 5H_2O$	0.25			
				$MnSO_4 \cdot H_2O$	2.50			
4	L-tyrosine	50		$ZnCl_2$	0.12			
				D-glucose 2.5 grams				
5	Choline chloride	50		Water to 50 ml.				

[a] The constituents of Nos. 1 and 2 were each present in a volume of 10 ml., 3 and 4 each in 5 ml., 5–10 and 12 each in 1 ml., and 11 in 25 ml.
[b] Polyoxyethylene sorbitan monooleate.

medium is presented in table 1. Most of the nutrients were prepared in solutions, and appropriate aliquots were measured out as needed. With the exception of L-cystine and L-tyrosine, which were dissolved with the aid of concentrated hydrochloric acid and 2M potassium hydroxide, respectively, the nutrients were dissolved or suspended in distilled water. Other combinations and concentrations of these substances can be used as long as the solubility of individual components is not exceeded. Aseptic techniques were used throughout the preparations. The medium was mixed in two parts; one was sterilized by filtration, the other by heat.

In preparation of the filtered portion the amino acid, vitamin, choline, sodium chloride, sodium and potassium phosphates, and magnesium sulfate solutions were mixed in the order given in table 1; the glucose then was added and the solution diluted to 48 ml. The pH was about 6.1 to 6.2 at this point. The mixture then was filtered through a Seitz filter. Toward the end of the filtration the solutions containing the iron, copper, manganese, and zinc salts were filtered, thus making a final volume of 50 ml. This solution was set aside until the second part was ready.

The heat-sterilized part of the medium was prepared in the following way. The corn oil, Tween 80, and cholesterol were weighed and placed in a 250-ml. Erlenmeyer flask and the α-tocopherol was added as a solution of 10 mg. in 1 ml. of ethanol. Several milliliters of ethyl ether were added until the lipides were just dissolved. The ether then was removed with an aspirator and 25 ml. of water were added. A larger quantity of this lipide mixture can be made up and stored in the refrigerator and measured out as needed. The potassium iodide solution, calcium carbonate, agar, and water, were added, and the flask was stoppered with cotton and autoclaved for 15 minutes at 15 pounds' pressure. After autoclaving, the sterile-filtered solution was poured into the hot mixture and rotated gently to insure homogeneous mixing. The medium was poured immediately under aseptic conditions into a hot sterile funnel the stem of which was equipped with a rubber tube closed with a pinch clamp. The funnel was heated by means of a Glas-Col heater within a range of 60° to 80° C. Two-milliliter portions of this hot medium were dispensed into sterile rearing vials and plugged with cotton.

The methods of collecting and preparing eggs, inoculation of vials, methods of observation, and interpretation of results have been reported previously (Vanderzant & Reiser 1956).

RESULTS.—Of 86 pink bollworms reared in three experiments, 73 became normal adults, 3 failed to emerge from their pupae, and 10 larvae remained in the resting stage. The length of the larval period was 16 to 24 days, which can be considered normal. The sizes of the pupae were also normal. Twenty-six female pupae weighed an average of 18.4 mg. with a range of 12.3 to 26.3 mg., and 41 males averaged 14.7 mg. with a range of 9.0 to 24.7 mg. Females oviposited a large number of fertile eggs. A second generation of adults was reared on the same medium. No further attempt was made to carry the insect over successive generations.

DISCUSSION.—The amino acid content of this medium was approximately 2 grams per 100 ml. The 10 essential amino acids comprised two-thirds of the amino acids, and the ratios of their amounts to each other were similar to those found in cottonseed as reported by Lyman *et al.* (1947). The amounts of the other amino acids, vitamins, choline, salts, and cholesterol were in excess of those previously found to be needed by the insect (Vanderzant 1956). Tween 80 was added as an emulsifying agent and α-tocopherol as an antioxidant. The agar was highly purified and had no nutritive value. The nutritive constituents of the medium were chemically defined except for the corn oil, which can be replaced by linoleic acid as previously reported (Vanderzant *et al.* 1956). When corn oil was replaced by linoleic acid with the concomitant omission of Tween 80 and α-tocopherol, the results were essentially the same as reported above. Therefore, a medium of known nutrient composition has been found that will promote the normal growth and reproduction of the pink bollworm. It is believed that this is the first insect to be perpetuated on a chemically defined diet.

REFERENCES CITED

Lyman, Carl M., Kenneth Kuiken, and Fred Hale. 1947. The essential amino acid content of cottonseed,

peanut and soybean products. Texas Agric. Expt. Sta. Bull. 692.

Vanderzant, Erma S. 1956. Unpublished data.

Vanderzant, Erma S., Dundappa Kerur, and Raymond Reiser. 1956. An insect assay for essential fatty acids. Presented at meeting of American Chemical Society, Dallas, Tex., April 8–13.

Vanderzant, Erma S., and Raymond Reiser. 1956. Studies of the nutrition of the pink bollworm using purified casein media. Jour. Econ. Ent. 49: 454–8.

Control of Rice Stem Borer with Endrin[1]

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The spring rice crop in Orissa, India is attacked by several insect pests of which the stem borer, *Schoenobius incertulas* (Walk.),[3] is considered the most destructive because it attacks the crop from nursery to maturity, prevailing throughout the season. There has been no recognized method of controlling this pest. In some instances 19.5% endrin emulsion concentrate has been reported as effective in controlling this pest. Cole (1955) reported that the minimum effective dosage of endrin for control of a rice stem borer had not been determined even though much field work had been conducted in Japan, Taiwan, and the Philippines. Muto & Takahasi (1954) reported on the use of different dosage rates of endrin against both generations of the asiatic rice borer, *Chilo suppressalis* (Wlk.), attacking rice at the Gifu Prefecture Agricultural Experiment Station where a high degree of control was obtained by spraying at or shortly before the peak of moth activity and again about 7 to 10 days later. The results of trials carried out using different dosages of endrin (0.13 lb. to 0.38 lb. per acre) against *Schoenobius incertulas* attacking rice at the State Agricultural Research Station, Bhubaneswar during 1955 are reported in this paper.

METHODS AND MATERIALS.—The rice variety D.I.3 was grown in the experimental field under the standard cultivation practices of the locality. The cultural and manurial operations were identical for treated and untreated plots, each of which was 121 square rods in area. There were four replications of each treatment and all plots were arranged in randomized fashion. An emulsion concentrate containing 19.5% of endrin was applied at the rate of 12, 16, or 32 ounces per acre which resulted in the application of 0.13, 0.19, and 0.38, pound respectively, of active toxic ingredient per acre. Two applications were made during the peak period of stem borer attack as determined by the previous history and by current observations of moth activity, egg masses, adult moth and initial dead heart counts. The dates fixed for the planted crop spray schedule were: February 4, first application—4 weeks after planting, and February 27, second application—about 3 weeks later.

RESULTS.—The results of these studies are presented in table 1.

CONCLUSION.—Endrin sprayed in doses of 0.13 to 0.19 pound per acre (12 to 16 oz. of 19.5% endrin emulsion concentrate) applied twice to the growing rice crop gave protection against attack by the rice stem borer. A dosage of 0.38 pound of endrin per acre gave still better results. The most important factor in obtaining good control of stem borer was correct timing of the applications.

Table 1.—Effect of the application of endrin sprays to the growing rice crop for control of *Schoenobius incertulas*, Bhubaneswar, India, 1955.

Endrin per Acre (Lb.)	Number per Square Yard[a]			Moth Activity
	Tillers	Stem Borer Dead Hearts	Egg Masses	
	Before first application			
0.13	305	49	23	Very high
.19	298	42	20	Very high
.38	295	31	25	Very high
None	297	25	23	Very high
	15 Days after first application			
0.13	322	12	7	Low
.19	338	9	7	Low
.38	353	7	4	Low
None	317	31	12	High
	Before second application			
0.13	388	13	9	Low
.19	413	11	8	Low
.38	421	9	6	Low
None	334	39	15	Very high
	15 days after second application			
0.13	399	6	3	Low
.19	441	7	4	Low
.38	462	3	2	Low
None	338	45	19	Very high

[a] Average of four replications in each case; 49 clumps per square yard.

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[2] The authors were assisted in the field work by D. Misra, Field Assistant.

[3] Lepidoptera, Schoenobiidae.

REFERENCES CITED

Cole, Leyland L. W. 1955. Endrin—World crops 7(4): 156. London.

Muto, Toshiro, and Fumion Takahasi. 1954. Rice stem borer (*Chilo suppressalis*) control with endrin. Shell Agric. Bull. ADB: 419/FC 15, pp. 1–4.

Effect of Soil-Type and DDT on Ovipositional Response of *Chrysopa californica* (Coq.) on Lemon Trees[1]

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During a population study of the citrus red mite, *Metatetranychus citri* (McG.), on six 3-year-old experimental lemon trees, it was observed that a natural population of the green lacewing, *Chrysopa californica* (Coq.), a predator of this mite, consistently laid more eggs on some trees than on others.

The six experimental trees were developed from cuttings taken at one time from a single parent tree. They were thus presumably genetically identical and were all of the same age. They were growing side by side in 32-gallon drums in an out-of-doors experimental plot at Riverside, California. Three of the trees were growing in a loose, sandy soil taken from the Utt lemon grove at Oxnard, California, and three were growing in a compact silt soil taken from the Perry lemon grove at Fillmore, California. One tree in each set had had 0.64 ounce of 50% wettable DDT added to the soil the first of each month from June through December, 1955.

A study of the mites and predators on these trees showed that each night the green lacewings laid more eggs on the three trees growing in the "Utt" soil than they did on the three trees growing in the "Perry" soil. Moreover, in each set they laid more eggs on the tree growing in the soil to which DDT had been

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Table 1.—Effect of soil type and DDT treatment on number of eggs deposited by the green lacewing on six experimental lemon trees, from June 15 through October 12, 1956.

Treatment	Number of Eggs Laid on Three Trees	
	In "Perry" Soil	In "Utt" Soil
Check	62	153
DDT added to soil	146	248
DDT sprayed on tree	178	199
Grand total	386	600

added than they did on the trees growing in untreated soil. Changing the relative positions of the trees within the plot did not alter this ovipositional pattern.

In order to gain more precise information on this phenomenon the relative influence of these six experimental lemon trees on ovipositional responses of *Chrysopa* was closely studied from February 3 to October 12, 1956. During most of this period the trees were freed of all mite and insect pests. Thus there were no mites, insects, or honeydew present to influence the reaction of the green lacewings.

In order to test further the influence of DDT in this matter, one tree in each set was treated with a DDT spray (2 lbs. 50% wettable DDT/100 gals.) on June 15 and again on August 3, 1956. Therefore, one tree of the three in each soil type was untreated, one was sprayed with DDT, and one had previously had DDT added to the soil.

Table 1 shows the total number of green lacewing eggs laid on each tree during the experimental period. A grand total of 386 green lacewing eggs was deposited on the three trees in the "Perry" soil, and of 600 eggs on the three trees in the "Utt" soil. Table 1 also shows that untreated (check) trees received fewer eggs than trees treated with wettable DDT, whether the DDT material was added to the soil or sprayed on the tree.

These studies are being continued in an attempt to determine the factor or factors responsible for this variation in ovipositional responses.

Insecticide Residues in Wireworm Control[1]

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Several insecticides will kill wireworms at a reasonable cost and without phytotoxic or other undesirable effects. After they have been killed, damaging populations can be re-established only by the survival of wireworms that hatch from eggs deposited by beetles from other fields. This will take several years, the number depending upon the influx of beetles and the time toxic residues remain in the soil. In the irrigated lands of the Pacific Northwest all the principal wireworm species emerge from the soil in the spring to mate, and then burrow back in the same field and lay most of their eggs. Most of them emerge again for a dispersal flight to find a suitable field in which to lay the remainder of their eggs. This flight is not over 3 or 4 miles. If a chemical that is toxic to young wireworms is present when the eggs are laid in cleaned fields, reinfestation cannot become established.

Materials and Methods.—In the fall of 1953 an experiment was begun to find out how long various insecticides applied to the soil would remain toxic to wireworms. The field selected was near Prescott, Washington, in the Touchet River valley. The soil there is of the Walla Walla silt-loam type, deep and well drained, and the rainfall totals about 15 inches a year, occurring mostly from November to May. Irrigation is needed in the late spring and summer months for most crops, although certain grains frequently can be grown without irrigation. This field was heavily infested with wireworms, principally *Limonius californicus* Mann., but a few *L. canus* Lec. and *Ctenicera pruinina noxia* (Hyslop) were present.

Quarter acre plots were established, each treated with one of the following chemicals and dosages per acre: aldrin at 3 and 6 pounds, chlordane at 4 and 8 pounds, dieldrin and heptachlor at 2 and 4 pounds, DDT at 10 and 20 pounds. The plots were arranged in four randomized blocks. Eight check plots were left untreated, two to each block. Soil samples for bioassay in the laboratory were taken from each plot 1 and 6 months and 1 and 2 years after treatment. Each sample was a composite of five subsamples taken at random near the center of the plot, thoroughly mixed and placed in two 6-ounce salve tins. In each tin can 10 medium to large wireworms were placed with four kernels of field corn that had been killed by hot water. Observations were made at 3-week intervals for 3 months and the dead wireworms removed.

Results.—The results are shown in table 1. After 1 month the soil from the plots containing both dosages of heptachlor, aldrin, and dieldrin and DDT at 20 pounds per acre gave kills of 85% or more. DDT at 10 pounds and chlordane at 8 pounds gave kills between 70 and 85%. Chlordane at 4 pounds gave about the same kill as the untreated checks. Some of these low kills were probably due to poor initial mixture of the chemical in the soil.

After 1 year heptachlor at both dosages, aldrin at 6 pounds, DDT at 20 pounds, dieldrin at 4 pounds, and chlordane at 8 pounds per acre all had highly toxic residues. The lower dosages of DDT, aldrin, dieldrin, and chlordane were inferior.

At the end of 2 years the soil treated with heptachlor at 4 pounds per acre continued to kill the greatest number of wireworms. Dieldrin at the same rate was also very effective. The plots receiving both dosages of DDT, the higher dosage of chlordane, and the low dosage of heptachlor all gave satisfactory kills. Aldrin, even at the highest dosage, and the low dosage of chlordane were inferior to the rest. In the soils treated with the low dosages of aldrin and chlordane the kills were not significantly better than in the untreated checks.

Table 1.—Per cent mortality of medium to large wireworms exposed for 3 months in soil taken at various times after treatment with insecticides in the fall of 1953.

Insecticide	Pounds per Acre	1 Month	6 Months	1 Year	2 Years
Heptachlor	4	100	100	99	94
	2	96	95	90	79
Aldrin	6	95	90	96	74
	3	91	94	81	61
DDT	20	89	88	95	82
	10	71	88	86	80
Dieldrin	4	85	94	93	90
	2	85	90	86	76
Chlordane	8	81	99	90	82
	4	56	96	81	54
Untreated checks:					
A	—	57	87	76	48
B	—	42	76	68	47
L.S.D. at 5% level		18	7	11	18

Newly hatched larvae are much easier to kill than older ones. For this reason reinfestation is delayed by the presence of insecticide residue in the soil even though the quantity is well below that necessary to kill larger larvae.

In the spring of 1955 tests were conducted with newly hatched larvae in soil from the Prescott plots to check the possibility of

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Table 2.—Survival of newly hatched wireworms from 400 eggs held for 60 days in samples of soil taken from plots 1½ years after being treated with insecticides.

Insecticide	Pounds per Acre	Number of Larvae	Insecticide	Pounds per Acre	Number of Larvae
Untreated checks:					
A	—	77	Dieldrin	4	0
B	—	73		2	1
Aldrin	6	0	DDT	20	0
	3	6		10	26
Chlordane	8	0	Heptachlor	4	0
	4	0		2	0
L.S.D. at 1% level				—	33
5% level				—	24

reinfestation. This was 1½ years after the soil had been treated with the insecticides. Soil samples were taken from the plots in the same manner as before, and 100 wireworm eggs, just about to hatch, were put in each can and incubated at 70° F. Ten kernels of killed wheat were added to each can, and supplementary food was added at the end of 30 days. After 60 days the soil from each can was washed, and the residue examined for larvae. As shown in table 2, all the treatments were markedly effective. DDT at 10 pounds, aldrin at 3 pounds, and dieldrin at 2 pounds per acre were the only ones that did not kill all the wireworms.

Heptachlor at 4 pounds per acre gave the best control of wireworms in these studies. At 2 pounds its effectiveness seems to be limited to 2 years, although reinfestation would probably be prevented for a longer period. Aldrin, even at 6 pounds per acre, cannot be depended upon to kill wireworms for more than 2 years, and 3 pounds for more than 1 year, although reinfestation will be prevented for a somewhat longer time. DDT is slow to act, but toxic residues last for several years. Dieldrin is comparable to heptachlor, although a little less effective at the same dosages. Chlordane at 8 pounds is slightly less effective than dieldrin, but at 4 pounds it is little better than the check. However, its toxicity to newly hatched larvae is sufficient to prevent reinfestation for at least 2 years, even at 4 pounds per acre. Studies with newly hatched larvae show clearly that reinfestation will be prevented even after the toxic level of the insecticide is below that necessary to kill larger wireworms. If advantage is taken of this fact and retreatment is delayed, the danger of building up undesirable residues in the soil will be greatly reduced. Retreatment should never be made until reinfestation takes place and there is again a danger of crop damage.

A Study of a Flow Meter for Spray Machines

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Users of spray machines in general and experimenters in particular have needed for a long time some quick method of determining rate of flow. The present discussion is not intended to provide the final answer, but is merely a report on one type of instrument. The principle involved in the instrument (known as a linameter) is relatively simple. It consists of a heavy stainless steel float which raises and lowers a magnetized steel ball in a slender pipe as the spray passes through. A magnet on the outside of the pipe operates the registering mechanism. Cost of the instrument when purchased 3 years ago was $316.00. Simpler and less expensive instruments are available.

The flow meter shown in operation in figure 1 has been used for 2 years. The performance of a 10-year-old hydraulic sprayer, has been studied with it. The accuracy of the instrument has been checked frequently by spraying into a steel barrel and measuring the quantity of spray delivered. The following information derived from our study of this flow meter may pe pertinent.

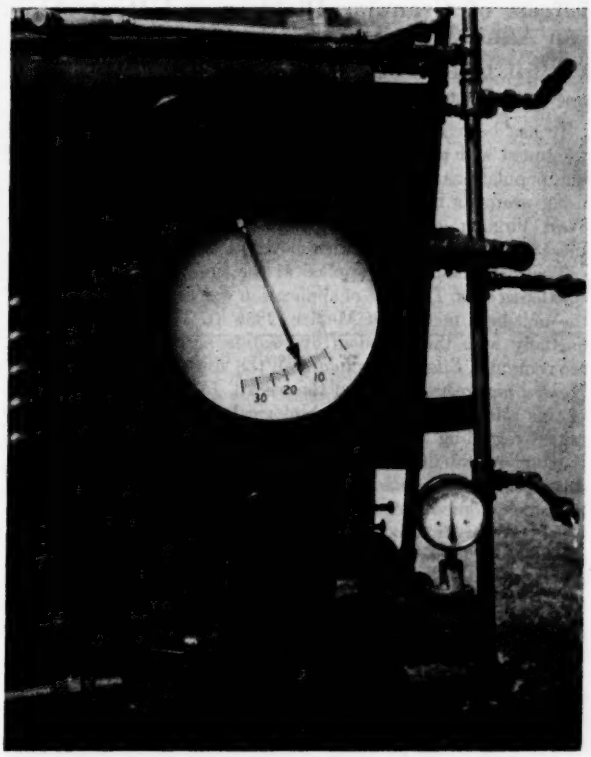

FIG. 1.—The flow-meter in operation showing a delivery of just under 15 gpm at about 500 pounds per square inch.

Table 1.—Pressure as related to delivery in the machine studied.[a]

Pressure (p.s.i.)	G.P.M. by Meter
600	10
500	14
500	15
500	19
475	20
375	22
200	25
000	30

[a] Engine speed 2300–2600 r.p.m. Pump speed 600 r.p.m. Pump capacity rated at 35 g.p.m.

(1) The principle is useful on experimental machines.
(2) The rated gallonage for a 10-year pump cannot be maintained without serious loss of pressure.
(3) Vibration is a serious limitation so that the instrument must be kept shut off when not in use.
(4) The air domes must be located between the pump and the instrument (applies to piston-type pumps).
(5) Accuracy with the particular instrument studied is greater at the higher gallonages.
(6) Provision for flushing and draining the meter is highly essential.

The relation of pressure to delivery is shown in table 1. Satisfactory operation in spraying large apple trees should not be over 20 gallons per minute.

Effects of Granular Dieldrin and Heptachlor on Adult Weevil Populations in Red Clover[1]

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It has been reported (Medler 1955 b) that pre-emergence treatment with granular insecticides will reduce meadow spittlebug populations. In connection with pre-emergence treatments using granular insecticides against the meadow spittlebug in West Virginia, observations were also made on the effect of these materials on several other clover pests.

The insecticides were applied at the time of the hatching of spittlebug eggs. The time of application was determined by using the unit-heat method of Medler (1955 a). This time coincides partially with the period of oviposition activity of the clover root curculio, *Sitona hispidula* (F.), the lesser clover leaf weevil, *Hypera nigrirostris* (F.) and the weevil, *H. meles* (F.). Because of this coincidence of similar biological activity, data were obtained regarding the effect of the insecticides on these weevils that are repeatedly in contact with the surface of the ground during their oviposition activity.

The experiment involved three treatments and an untreated check. The treatments and check were replicated four times in randomized ½-acre plots. Granular formulations of heptachlor and dieldrin were applied April 26, 1956, using a fertilizer-type spreader at the rate of 0.5 lb. actual insecticide per acre. Heptachlor emulsifiable concentrate was applied May 3, 1956, by a ground sprayer at the rate of 6 oz. actual heptachlor per acre. Counts were made (100 sweeps with an insect net per plot) on May 8, 18 and 29. The field was scheduled for cutting June 1. The results of the counts of adult weevils are shown in table 1.

The oviposition period of these weevils varies and extends over a period of several months. *Sitona hispidula* adults lay some eggs in the fall, but the majority of them are deposited in the spring. The adults overwinter in the field becoming active on warm days in the early spring. *Hypera nigrirostris* and *H. meles* also overwinter as adults and are active in the early spring in the clover fields. The excellent adult control obtained of *S. hispidula* was probably due to their movements on the surface of the ground during egg laying and their habit of dropping to the ground and feigning death when disturbed while feeding on foliage.

There was no obvious reason for the difference in control obtained of the two species of *Hypera* by granulated insecticide treatments. The total number of *H. meles* collected in 1600 sweeps on each of the dates indicates that the adults became progressively more abundant. These totals were 84, 146, and 205, respectively. The increasing numbers collected from the insecticide-treated plots on succeeding dates indicates the probability that the plots were being invaded by adults of this species from outside fields.

No effect attributable to insecticide action was observed in the populations of lygus bugs or leafhoppers that subsequently developed in the field.

SUMMARY.—Populations of adult *Sitona hispidula* and *Hypera nigrirostris* were reduced in excess of 90% by an early season application of granules of dieldrin or heptachlor to the surface of the ground at the rate of 0.5 lb. actual insecticide per acre. *Hypera meles* adult populations were not effectively reduced by these treatments.

REFERENCES CITED

Medler, J. T. 1955a. Method of predicting the hatching date of the meadow spittlebug. Jour. Econ. Ent. 48(2) 204–5.

Medler, J. T. 1955b. Meadow spittlebug control by pre-emergence treatment. Jour. Econ. Ent. 48(5) 593–5.

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Control of Corn Earworm in Sorghum Heads by Aerial Spraying in Southwestern Kansas[1]

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The corn earworm, *Heliothis zea* (Boddie), has been an important pest attacking grain sorghum heads in Kansas for many

[1] Contribution No. 676, Department of Entomology and No. 11, Garden City Kansas Branch, Kansas Agricultural Experiment Station, Manhattan. Accepted for publication January 28, 1957.

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Table 1.—Effect of some insecticide treatments on populations of adult weevils in red clover plots. Huttonsville, West Virginia, 1956.

INSECT AND TREATMENT	ADULTS FROM ALL PLOTS[a]			AVERAGE NUMBER ADULTS PER 100 SWEEPS			OVERALL AVERAGE PER 100 SWEEPS	% POPULATION REDUCTION
Sitona hispidula	May 8	May 18	May 29	May 8	May 18	May 29		
Untreated	154	226	194	38.5	56.5	48.5	47.8	0
Dieldrin granules	3	3	18	0.8	0.8	4.5	2.0	95.8
Heptachlor granules	1	4	5	0.3	1.0	1.3	0.8	98.3
Heptachlor spray	4	1	4	1.0	0.3	1.0	0.8	98.4
Hypera nigrirostris								
Untreated	50	71	64	12.5	17.8	16.0	15.4	0
Dieldrin granules	6	3	4	1.5	0.8	1.0	1.1	92.9
Heptachlor granules	0	1	6	0.0	0.3	1.5	0.6	96.2
Heptachlor spray	0	0	1	0.0	0.0	0.3	0.1	99.5
Hypera meles								
Untreated	47	58	48	11.8	14.5	12.0	12.8	0
Dieldrin granules	24	55	82	6.0	13.5	20.5	13.3	—[b]
Heptachlor granules	11	25	41	2.8	6.3	10.3	6.6	48.4
Heptachlor spray	2	8	34	0.5	2.0	8.5	3.7	71.3

[a] Based on 100 sweeps from each of four replications.
[b] 4.35% increase.

years. It was quite abundant in several sorghum fields examined in Haskell County during the summer of 1956. Some of the fields had 60 to 80% of the heads infested with 1 to 4 larvae per head. Burkhardt *et al.* (1955) reported many fields in north-central Kansas had from 40 to 60% of the sorghum heads infested with larvae ranging from 1 to 8 per head.

Although numerous investigations have been conducted against this pest attacking corn, very little information is available regarding its chemical control in sorghum heads. Recent studies by Burkhardt *et al.* (1955) have indicated that the highest percentage control at the end of 24 hours by ground application was obtained with Phosdrin (96%) followed by DDT (84%). Heptachlor and Shell 1808 (2-carbethoxy-1-methylvinyl diethyl phosphate) gave the lowest percentage control with 41 and 37%, respectively. Dahms *et al.* (1955) reported that aldrin and a DDT-TEPP combination gave complete control of the corn earworm by spraying but chlordane, BHC, TEPP, and demeton were ineffective at the dosage used.

Recommended control for the corn earworm in sorghum heads has been an application of DDT at the rate of 2 to 3 pounds actual toxicant per acre. However, the use of DDT presents a residue problem. Therefore, it was the objective of this study to find some effective compounds to replace DDT.

MATERIALS AND METHODS.—Aerial applications were made near Sublette, Kansas on September 10, 1956, as follows: Parathion, 0.5 lb.; DDT, 2.0 lbs.; malathion. 1.0 lb.; endrin, 0.5.; and Phosdrin, 0.5 lb. per acre. All sprays were prepared from emulsifiable concentrates. Application was made by a Stearman biplane equipped with a 450-horsepower engine and a constant speed propeller. The spray system consisted of an interior supply tank; a centrifugal pump mounted below the fuselage equidistant between the landing gear struts and powered by an 18-inch, wind-driven, 6-bladed metal propeller; and a 40-foot boom with 12 nozzle outlets spaced equidistant along its entire length. The materials were applied at the rate of 3 gallons of total emulsion per acre and at a flight level of 6 to 12 inches above the plants. Wind velocity was estimated at 5 to 8 miles per hour during application. Temperature was 76° F., but reached a maximum of 101° F. during the day. No precipitation occurred during the study.

Because aerial equipment was used in this investigation, each treatment included 20 acres or more and was not replicated.

Density of earworms was determined by recording larvae present in 30 randomly selected sorghum heads in each plot. Pretreatment sampling revealed no significant variation in infestation throughout the field. Percentage control was based on differences in larval counts between the treated and check plots.

RESULTS AND DISCUSSION.—Results are presented in table 1. Phosdrin was considerably better than any of the other insecticides tested, giving 95% control 1 day after application. Satisfactory control (83%) was obtained with DDT followed closely by malathion (81%). Endrin and parathion gave the lowest control with 74 and 71%, respectively. It was not determined whether mortality from Phosdrin resulted from contact, fumigant, or systemic action.

Table 1.—Effectiveness of several insecticides for control of corn earworm attacking sorghum heads. Sublette, Kansas. 1956.

Treatment	Pounds Actual Toxicant per Acre	Number of Larvae After (Days)		Per Cent Reduction of Larvae After (Days)	
		1	4	1	4
Phosdrin[a]	0.5	2	0	95	100
DDT	2.0	7	3	83	92
Malathion[b]	1.0	8	4	81	89
Endrin[c]	0.5	11	2	74	94
Parathion	0.5	12	5	71	86
Check	—	42	36	—	—

[a] Supplied by Shell Chemical Company.
[b] Supplied by American Cyanamid Company.
[c] Supplied by Velsicol Chemical Corporation.

At the end of 4 days after application, Phosdrin provided 100% control; endrin and DDT, 94 and 92%, respectively; malathion, 89%; and parathion, 86%, the lowest control. Except for malathion, the results corroborated those of Burkhardt *et al.* (1955).

SUMMARY.—Aerial spray tests for the control of the corn earworm, *Heliothis zea* (Boddie) in the heads of grain sorghum were conducted in southwestern Kansas during the summer of 1956. The materials used and dosages applied were: parathion, 0.5 lb.; DDT, 2.0 lbs.; malathion, 1.0 lb.; endrin, 0.5 lb.; and Phosdrin 0.5 lb. actual toxicant per acre. Percentage control obtained with these formulations after 1 and 4 days, respectively, were: Phosdrin, 95 and 100; DDT, 83 and 92; and malathion, 81 and 89. Although endrin at 0.5 lb. and parathion at 0.5 lb. did not give appreciable control after 1 day, the 4-day count resulted in 94 and 86% control, respectively.

REFERENCES CITED

Burkhardt, C. C., and M. P. Breithaupt. 1955. Chemical control of the corn earworm in sorghum heads. Jour. Econ. Ent. 48(2): 225.

Dahms, R. G., J. B. Sieglinder and W. D. Guthrie. 1955. Methods of treating sorghum selfing bags for insect control. Jour. Econ. Ent. 48(5): 568–72.

Common Names of Insecticides

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This is a complete revision of the list of common names of insecticides compiled by the Committee on Insecticide Terminology of the Entomological Society of America (of which the author is chairman) for use in the JOURNAL OF ECONOMIC ENTOMOLOGY. It supersedes the list issued in February 1955. This list has also been adopted for use in the Agricultural Research Service, U. S. Department of Agriculture.

Like its predecessors, this list is confined to the principal organic compounds that have been developed as insecticides in recent years, the chemical names of which are too long for convenient use. In addition to their chemical definitions, other designations that have been given to them in the literature and elsewhere are shown. This revision includes many new materials and some changes to conform with the latest approved usage. The chemical definitions of some of the materials, particularly the organic phosphorus compounds, have been changed to agree with Chemical Abstracts nomenclature.

Insecticides with common names that have been approved by the Interdepartmental Committee on Pest Control (indicated by*) shall be called by those names. Some other materials are best known by their proprietary names or company numbers, and their use seems to be justified if they are not competitive products; such names are capitalized. Other materials have designations that have become so firmly established through continued use that a change does not seem in order. These names, given in the left-hand column of the list, may be used with no explanation as to their chemical composition.

NAME TO BE USED	CHEMICAL DEFINITION	OTHER DESIGNATIONS THAT HAVE BEEN USED
*aldrin	not less than 95% of 1,2,3,4,10,10-hexachloro-1,4,4*a*,5,8,8*a*-hexahydro-1,4-*endo-exo*-5,8-dimethanonaphthalene	compound 118
*allethrin	*dl*-2-allyl-4-hydroxy-3-methyl-2-cyclopenten-1-one esterified with a mixture of *cis* and *trans dl*-chrysanthemummonocarboxylic acid	allyl homolog of cinerin I synthetic pyrethrins
Am. Cyanamid 4124	*O*-(2-chloro-4-nitrophenyl)*O,O*-dimethyl phorphorothioate	
Am. Cyanamid 12008	*O,O*-diethyl *S*-(isopropylthio)methyl phosphorodithioate	
Am. Cyanamid 12009	*O,O*-diethyl *S*-(propylthio)methyl phosphorodithioate	
Am. Cyanamid 12415	*O,O*-diethyl *O*-[6-(3(2H)-pyridazinonyl)] phosphorothioate	
Am. Cyanamid 12433	*O,O*-dimethyl *O*-[6-(3(2H)-pyridazinonyl)] phosphorothioate	
Am. Cyanamid 12503	*O,O*-diethyl *O*-[6-(3(2-phenyl)-pyridazinonyl)] phosphorothioate	
Am. Cyanamid 18682	*O,O*-diethyl *S*-(*N*-isopropylcarbamoyl)-methyl phosphorodithioate	
Aramite	2-(*p-tert*-butylphenoxy)-1-methylethyl 2-chloroethyl sulfite	compound 88R alkyl aryl sulfite
azobenzene	azobenzene	azobenzide diphenyl diimide
Bayer 21/199	*O*-(3-chloro-4-methylumbelliferone) *O,O*-diethyl phosphorothioate	
Bayer 21/200	*O*-(3-chloro-4-methylumbelliferone) *O,O*-dimethyl phosphorothioate	
Bayer 28/63	[2-(diethoxyphosphinylthio)ethyl]-ethyl methyl sulfonium methyl sulfate	
Bayer 16259	*O,O*-diethyl *S*-(4-oxo-3H-1,2,3-benzotriazine-3-methyl) phosphorodithioate	benzotriazine deriative of an ethyl dithiophosphate
Bayer 19639	*O,O*-diethyl *S*-2-(ethylthio)ethyl phosphorodithioate	Di-syston
BHC	1,2,3,4,5,6-hexachlorocyclohexane, consisting of several isomers and containing a specified percentage of gamma	benzene hexachloride gammexane
Bulan	1,1-bis(*p*-chlorophenyl)-2-nitrobutane	CS-674A
butoxy polypropylene glycol	butoxypropanediol polymer	Crag Fly Repellent
Chipman R-6199	*O,O*-diethyl *S*-(2-diethylamino)ethyl phosphorothioate [hydrogen oxalate salt]	Tetram
Chlorbenside	*p*-chlorobenzyl *p*-chlorophenyl sulfide	Mitox
Ch orobenzilate	ethyl 4,4′-dichlorobenzilate	Geigy 338 G-23992
*chlordane	1,2,4,5,6,7,8,8-octachloro-2,3,3*a*,4,7,7*a*-hexahydro-4,7-methanoindene	chlordan Velsicol 1068 Octa-Klor Octachlor
Chlorthion	*O*-(3-chloro-4-nitrophenyl)*O,O*-dimethyl phosphorothioate	Bayer 22/190
cyclethrin	*dl*-2-(2-cyclopentenyl)-4-hydroxy-3-methyl-2-cyclopenten-1-one esterified with a mixture of *cis* and *trans dl*-chrysanthemummonocarboxylic acids	
D-D mixture	mixture of 1,2-dichloropropane and 1,3-dichloropropene	D-D Dowfume N
DDT	1,1,1-trichloro-2,2-bis(*p*-chlorophenyl)ethane	chlorophenothane dichloro-diphenyltrichloroethane
DDVP	2,2-dichlorovinyl dimethyl phosphate	
demeton	mixture of *O,O*-diethyl *S* (and *O*)-2-(ethylthio)ethyl phosphorothioates	a trialkyl thiophosphate Systox
Diazinon	*O,O*-diethyl *O*-(2-isopropyl-4-methyl-6-pyrimidinyl) phosphorothiate	G-24480
dichloroethyl ether	bis(2-chloroethyl) ether	
*dieldrin	not less than 85% of 1,2,3,4,10,10-hexachloro-6,7-epoxy-1,4,4*a*,5,6,7,8,8*a*-octahydro-1,4-*endo-exo*-5,8-dimethanonaphthalene	compound 497 Octalox HEOD

NAME TO BE USED	CHEMICAL DEFINITIONS	OTHER DESIGNATIONS THAT HAVE BEEN USED
Dilan	mixture of 1 part of Prolan and 2 parts of Bulan	CS-708
dimefox	tetramethyl phosphorodiamidic fluoride	Hanane Pestox 14
dimetan	dimethyl 3-hydroxy-5,5-dimethyl-1-oxo-2-cyclohexenyl carbamate	G-19258
dimethyl carbate	dimethyl *cis*-bicyclo[2.2.1]hept-5-ene-2,3-dicarboxylate	
Dimite	4,4′-dichloro-α-methylbenzhydrol	di(*p*-chlorophenyl) methyl-carbinol 1,1-bis(*p*-chlorophenyl)ethanol DMC
dinitrobutylphenol	4,6-dinitro-*o*-*sec*-butylphenol	Dinoseb DNOSBP
dinitrocyclohexyl-phenol	4,6-dinitro-*o*-cyclohexylphenol	DNOCHP Dinex
dinitrocresol	4,6-dinitro-*o*-cresol	DNOC DNC Sinox
Dipterex	*O,O*-dimethyl 2,2,2-trichloro-1-hydroxyethylphosphonate	Bayer L 13/59
Dow ET-15	*O*-methyl *O*-(2,4,5-trichlorophenyl)phosphoramidothioate	
Dow ET-57	*O,O*-dimethyl *O*-(2,4,5-trichlorophenyl)phosphorothioate	
*endrin	1,2,3,4,10,10-hexachloro-6,7-epoxy-1,4,4*a*,5,6,7,8,8*a*-octahydro-1,4-*endo*-*endo*-5,8-dimethanonaphthalene	compound 269
EPN	*O*-ethyl *O*-*p*-nitrophenyl phenylphosphonothioate	EPN-300
ethyl hexanediol	2-ethyl-1,3-hexanediol	Rutgers 612
furethrin	*dl*-2-(2-furfuryl)-4-hydroxy-3-methyl-2-cyclopenten-1-one esterfied with a mixture of *cis* and *trans* *dl*-chrysanthemummonocarboxylic acids	
Genite	2,4-dichlorophenyl benzenesulfonate	Genitol 923
Guthion	*O,O*-dimethyl *S*-(4-oxo-3H-1,2,3-benzotriazine-3-methyl) phosphorodithioate	Bayer 17147 Gusathion
heptachlor	3*a*,4,5,6,7,8,8-heptachloro-3*a*,4,7,7*a*-tetrahydro-4,7-methanoindene	Velsicol 104 E-3314
Hercules AC-528	2,3-*p*-dioxanedithiol *S,S*-bis(*O,O*-diethyl phosphorodithioate)	
HETP	mixture of ethyl polyphosphates containing 12 to 20% of tetraethyl pyrophosphate	hexaethyl tetraphosphate
Indalone	butyl 3,4-dihydro-2,2-dimethyl-4-oxo-2*H*-pyran-6-carboxylate	*n*-butyl mesityl oxide oxalate butopyronoxyl
*isodrin	1,2,3,4,10,10-hexachloro-1,4,4*a*,5,8,8*a*-hexahydro-1,4-*endo*-*endo*-5,8-dimethanonaphthalene	compound 711
Isolan	dimethyl 5-(1-isopropyl-3-methyl-pyrazolyl) carbamate	G-23611
Karathane	2-(1-methylheptyl)-4,6-dinitrophenyl crotonate	
Kelthane	1,1-bis(*p*-chlorophenyl)-2,2,2-trichloroethanol	Rohm & Haas FW-293
lauseto neu	chloromethyl *p*-chlorophenyl sulfone	
Lethane 60	2-thiocyanoethyl laurate	
Lethane A-70	bis(2-thiocyanoethyl) ether	dithiocyanodiethyl ether
Lethane 384	2-(2-butoxyethoxy)ethyl thiocyanate	butoxy thiocyanodiethyl ether
*lindane	1,2,3,4,5,6-hexachlorocyclohexane gamma isomer of not less than 99% purity	gamma BHC
*malathion	*S*-[1,2-bis(ethoxycarbonyl)ethyl]*O,O*-dimethyl phosphorodithioate	diethyl mercaptosuccinic acid, *S*-ester with *O,O*-dimethyl phosphorodithioate malathon compound 4049
Malrin	a formulation containing 1.8% of malathion and 3.6% of Perthane	
Metacide	a formulation of methyl parathion and parathion	
*methoxychlor	1,1,1-trichloro-2,2-bis(*p*-methoxyphenyl)ethane	Marlate DMDT
methyl demeton	mixture of *O,O*-dimethyl *S*-(and *O*-) (2-ethylthio)ethyl phosphorothioates	meta-Systox
methyl parathion	*O,O*-dimethyl *O*-*p*-nitrophenyl phosphorothioate	methyl homolog of parathion
MGK 264	*N*-(2-ethyhexyl)bicyclo[2.2.1]hept-5-ene-2,3-dicarboximide	Octacide 264 Van Dyk 264
Neotran	bis(*p*-chlorophenoxy)methane	di(4-chlorophenoxy)-methane K-1875
ovex	*p*-chlorophenyl *p*-chlorobenzenesulfonate	K-6451 Ovotran
para-oxon	diethyl *p*-nitrophenyl phosphate	oxygen analog of parathion E-600
*parathion	*O,O*-diethyl *O*-*p*-nitrophenyl phosphorothioate	E-605 compound 3422 Thiophos Niran

NAME TO BE USED	CHEMICAL DEFINITIONS	OTHER DESIGNATIONS THAT HAVE BEEN USED
Perthane	1,1-dichloro-2,2-bis(*p*-ethylphenyl)ethane	Q-137
Phosdrin	1-methoxycarbonyl-1-propen-2-yl dimethyl phosphate, 60% technical	Shell OS-2046
Phostex	a mixture of bis(dialkyloxyphosphinothioyl) disulfides	Niagara 1137
piperonyl butoxide	α-[2-(2-butoxyethoxy)ethoxy]-4,5-methylenedioxy-2-propyltoluene	(butyl carbitol) (6-propylpiperonyl) ether
piperonyl cyclonene	mixture of 3-alkyl-6-carbethoxy-5-(3,4-methylenedioxyphenyl)-2-cyclohexene-1-one and 3-alkyl-5-(3,4-methylenedioxyphenyl)-2-cyclohexen-1-one	piperonyl cyclohexenone
Pirazinon	*O,O*-diethyl *O*-3-(2-propyl-6-methyl-4-pyrimidinyl)phosphorothioate	G-24622
Potasan	*O*-(4-methylumbelliferone)*O,O*-diethyl phosphorothioate	E-838
		O,O-diethyl thiophosphoric acid ester of 7-hydroxy-4-methylcoumarin
Prolan	1,1-bis(*p*-chlorophenyl)-2-nitropropane	CS-645A
propyl isome	dipropyl 5,6,7,8-tetrahydro-7-methylnaphtho[2,3]-1,3-dioxole-5,6-dicarboxylate	*n*-propyl isome
		dipropyl 3-methyl-6,7-methylenedioxy-1,2,3,4-tetrahydronaphthalene-1,2-dicarboxylate
Pyrolan	dimethyl 5-(3-methyl-1-phenylpyrazolyl) carbamate	G-22008
schradan	octamethyl pyrophosphoramide	bis(dimethylamino)phosphonous anhydride
		Pestox III
		OMPA
sesamin	2,6-bis(3,4-methylenedioxyphenyl)-3,7-dioxabicyclo[3.3.0] octane	
sesamolin	2-(3,4-methylenedioxyphenyl)-6-(3,4-methylenedioxyphenoxy)-3,7-dioxabicyclo[3.3.0]octane	
Shell OS 1836	2-chlorovinyl diethyl phosphate	
Strobane	a mixture of chlorinated terpenes with about 66% chlorine	compound 3961
sulfotepp	ethyl thiopyrophosphate	*O,O,O,O*-tetraethyl-dithiopyrophosphate
		dithione
		Sulfatep
sulfoxide	1,2-methylenedioxy-4-[2-(octylsulfinyl)propyl]benzene	Sulfox-Cide
		n-octyl sulfoxide of isosafrole
Sulphenone	*p*-chlorophenyl phenyl sulfone	R-242
TDE	1,1-dichloro-2,2-bis(*p*-chlorophenyl)ethane	DDD
		Rhothane
		tetrachlorodiphenylethane
Tedion	2,4,4′,5-tetrachlorodiphenyl sulfone, 80% technical	
TEPP	ethyl pyrophosphate, appro. 40% technical	tetraethylpyrophosphate
		TEP
Thanite	mixture of fenchyl and isobornyl thiocyanoacetates	terpinyl thiocyanoacetate
Thimet	*O,O*-diethyl *S*-(ethylthio)methyl phosphorodithioate	Am. Cyanamid 3911
Thiodan	6,7,8,9,10,10-hexachloro-1,5,5*a*,6,9,9*a*-hexahydro-6,9-methano-2,4,3-benzodioxathiepin-3-oxide	Niagara 5462
		Malix
*toxaphene	chlorinated camphene containing 67–69% of chlorine	compound 3956
Trithion	*S*-(*p*-chlorophenylthio)methyl *O,O*-diethyl phosphorodithioate	Stauffer R-1303

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OBITUARIES

Robert Kemble Fletcher
1884-1956

Dr. Robert K. Fletcher, Entomologist for 30 years with the Texas A. & M. College System, died at his home in Dallas, Texas, October 4, 1956, at the age of 72.

Dr. Fletcher was born August 6, 1884, in Curvinsville, Pennsylvania. He received his B.A. degree from the University of Maine in 1917 and his M.A. degree in 1919 and his Ph.D. degree in 1928 from Ohio State University. He was appointed Assistant Professor in the Department of Entomology at Texas A. & M. College in September, 1919, and was promoted to Associate Professor in 1920. During the years 1919 to 1927, he taught various entomological courses and was highly respected by his students as well as his co-workers.

In 1927 Dr. Fletcher transferred to a full-time research position with the Texas Agricultural Experiment Station. In this capacity, he was engaged in biological and control studies of the major insect pests of cotton. Although Dr. Fletcher did not publish extensively, the results of his studies, especially on the biology of the cotton bollworm, proved most valuable to the development of more efficient control measures. Dr. Fletcher was also much interested in a taxonomic study of the family Cicadellidae and published a number of papers relating to the Brazos County fauna. His interests extended into the field of ornithology and he was considered one of the best informed local men in this field and in other biological sciences related to entomology.

Dr. Fletcher was a member of Sigma Xi, American Association of University Professors, American Association of Economic Entomologists and a Fellow of the American Association for the Advancement of Science and of the Texas Academy of Science.

Dr. Fletcher is survived by his wife, Mrs. Ruth C. Fletcher, now living in Dallas, Texas. He will long be remembered by his associates for his leadership in the field of biology.

J. C. GAINES

Robert Kemble Fletcher
1884–1956

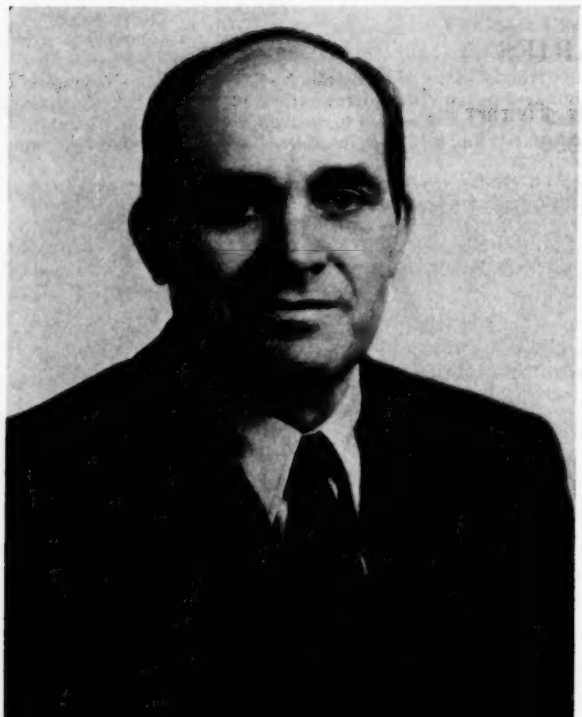
Harry Ray Bryson
1892-1956

Harry R. Bryson, Associate Professor of Entomology and Associate Entomologist of the Agricultural Experiment Station, Kansas State College, Manhattan, died December 3, 1956, after a long period of distressing physical affliction, at the age of 64. He was born, reared and educated in Kansas, having received his B.S. and M.S. degrees, the latter with Phi Kappa Phi honors, from Kansas State College. He was employed as a member of the Entomology staff of Kansas State College in 1924, at first to take the major responsibility for the experiment station project on underground insects, under the direction of the late Prof. J. W. McColloch. He continued in charge of that difficult and important research project to the time of his death. He was the author of several dozen scientific papers on such important insects as chinch bugs, sorghum insects, wireworms, false wireworms and white grubs.

Professor Bryson's greatest contribution was his superb teaching. He had had 5 years of successful experience as a high school teacher, and his ability to interest students in entomology and to make the courses stimulating to them was soon recognized. Consequently, he was given major responsibilities in the basic course in economic entomology, in which he continued until chronic illness required reduced activities.

He developed a course in Insect Ecology, which was elected by a sizable number of students every year it was offered. He had in preparation a full outline text which he had planned to finish for publication but, because of his illness, this was not quite completed. His emphasis was especially on soil ecology, which is a viewpoint not commonly used.

Professor Bryson's classes were alerted by his wit and pointed humor, locally known as "Brysonisms." He had a strongly prac-



tical emphasis in his teaching. It is common, in Kansas, for county agents, vocational agricultural teachers, and many others to remark that he was one of their best teachers in college.

Professor Bryson is survived by his wife, a son, William Ronald, in college, and a daughter, Gladys Kathleen, who is a junior in high school. His family meant much to him. He was a member of Sigma Xi and Gamma Sigma Delta, in addition to Phi Kappa Phi. He was a past president of the Kansas Entomological Society, and a long-time member of the former two national entomological societies.

The minister at the memorial service emphasized Professor Bryson's great effort and determination to do his work to the very last under his great physical handicaps. Many times, climbing the steps to his office required his maximum strength but, though exhausted and sometimes in pain, he tried desperately to carry on. This spirit of determination and his superb teaching ability continue as an inspiration to all who knew him.

ROGER C. SMITH

Harry Ray Bryson
1892–1956

Edward Walley Jones
1904-1955

The many friends of Edward Walley Jones were saddened by his passing in Twin Falls, Idaho, on August 3, 1955, at the age of 50.

He was a native of the Pacific Northwest, born in Portland, Oregon, on August 28, 1904; was educated in Portland grade and high schools; and was graduated from Reed College in that City in 1927 with an A.B. degree. His graduate work included studies at the University of Minnesota, from which he received an A.M. degree in 1930, at Iowa State College in 1931, and at the University of California in 1934.

Most of his professional life was also spent in the Pacific Northwest and Intermountain regions. His professional career as a research entomologist began in the summer of 1929 with a temporary appointment at the former U. S. Bureau of Entomology and Plant Quarantine field laboratory, at Walla Walla, Washington. He returned to this same laboratory on a permanent basis in 1930 and continued here in a professional capacity, with research work on the biology, ecology, and control of wireworms, until 1949. He was transferred to the Twin Falls, Idaho field laboratory of the former Bureau of Entomology and Plant Quarantine in 1949, where he continued his entomological career with research and control work on the beet leafhopper and the sugar-beet root maggot.

In 1951 he suffered a severe heart attack, which incapacitated him for further entomological research work, and he was retired from Federal service on disability in 1952. Although his condition improved sufficiently to permit him to take up other light, sedentary work during 1953 and 1954, he remained an entomologist at heart; and even during his latter months, he thoroughly enjoyed informal entomological discussions with friends who called.

In 1941, Mr. Jones was married to Rowena Smith in Walla Walla, Washington. He is survived by his widow and two daughters, Kathryn and Sharon, all of Twin Falls, Idaho; a brother Phillip and a sister Mrs. Gladys Cox, both of Portland, Oregon.

He was a member of a number of Masonic Lodge bodies and formerly had been very active in these organizations. He was a long-time member of the Prebyterian Church. He was also a member of the National Association of Retired Civil Employees and charter president of Twin Falls Chapter 406, an office he held at the time of his death. His scientific and professional affiliations included membership in the honorary scientific society Sigma Xi, American Association of Economic Entomologists, Entomological Society of America, Ecological Society, and the Pacific Entomological Society.

Mr. Jones was at all times sincere, energetic, and enthusiastic in planning and carrying out his work. Throughout his career in entomological work, he proved himself a devoted and conscientious public servant. He authored a number of publications on the biology, ecology, and control of wireworms and also was coauthor of one each on the control of the beet leafhopper and sugar-beet root maggot.

All his friends knew him as a man of integrity and high principles. In his personal life he lived quietly, devoted to his wife and family. His high ideals and friendly spirit won him many friends in both personal and professional life. He will be missed, and his passing is deeply regretted by all who knew him.

KENNETH E. GIBSON
J. R. DOUGLASS